cells differentiated along myeloid (acute promyelocytic) or monocytoid (acute myelomonocytic) pathways, their cholesterol-synthesis rates diverged and approached those of their respective mature cellular counterparts, the neutrophil or the peripheral blood monocyte. Enhanced sterol synthesis in leukemic cells could not be explained by more rapid efflux of membrane cholesterol to the environment. In addition, the different rates of cholesterol biosynthesis in leukemic-cell subgroups did not correlate with differences in their rates of cellular DNA synthesis. As could be seen from the material presented in this paper, the normal divergence of sterol-synthesizing capacity found between mature neutrophils and monocytes develops at an early stage of differentiation and is detectable even in leukemic cells.

Yachnin, S., Larson, R. A. and West, E. J.

British Journal of Haematology 54:459-466, 1983.

Other support: U. S. Public Health Service and the Nalco Cancer Research Center.

From the Department of Medicine and Committee on Immunology, the University of Chicago, Chicago.

IV. *Neuropharmacology and Physiology*

GENETICS OF NICOTINE RESPONSE IN FOUR INBRED STRAINS OF MICE

Nicotine, which is known to have multiple effects, appears to act at the neuromuscular junction, at the autonomic ganglia and in the brain. The actions of nicotine also appear to be influenced by genetic factors. In the present attempt to assess these genetic influences, the effects of nicotine on five behavioral and physiological measures were determined in four inbred mouse strains (BALB, C57BL, DBA and C3H). In addition, the binding characteristics of nicotine and α-bungarotoxin (α-BTX), two ligands which appear to label different nicotinic receptors, were measured in seven discrete brain regions as well as in whole brain. Results showed that a number of differences in response to nicotine were found among the four inbred strains. Whereas nicotine depressed open-field activity of BALB, C57BL and DBA mice in a dose-dependent manner, low doses of nicotine increased locomotor activity in C3H mice. The doses of nicotine tested reduced Rotarod performance in DBA and C57BL mice but not in C3H and BALB mice. All four strains displayed a dose-dependent decrease in body temperature after nicotine administration. The BALB mice were more sensitive to the agent than were the C3H, while the effects on C57BL and DBA mice were intermediate. All four strains showed a transient increase in respiration only after a high (2.0 mg/kg) nicotine dose. No dose of nicotine was found to have an effect on the startle response after auditory stimulation in three of the strains; only the C3H mice exhibited enhanced startle after nicotine administration. Measurement of the affinity and density of nicotine and α-BTX binding sites provided no clear explanation for the differences among the strains. The affinity of nicotine for either the DL-[^{3}H] or α-BTX binding sites did not vary from strain to strain or from region to region; however, several differences in the density of α-BTX binding sites were detected in the hippocampus and midbrain. Thus, genetic factors influence response to nicotine, but variation in response is not easily explained by differences in brain nicotinic receptors.

Marks, M. J., Burch, J. B. and *Collins,*

The Journal of Pharmacology and Expe

Other support: National Institutes of He

From the Institute for Behavioral Genet Colorado, Boulder.

QUANTITATION OF TOLERANCE D OXOTREMORINE TREATMENT

An earlier study concerning the effec clear evidence of the development of tole used to evaluate tolerance in that stud tolerance development. For the study pre to quantitate the tolerance which develo muscarinic agonist. Cumulative dose-res of oxotremorine on body temperature ar quential injections to individual animals. ably to those constructed by injecting indi sequential injection technique was used t ment to oxotremorine. A linear relationsh and magnitude of change of the ED_{50} valu observed, with animals receiving an infu increase in ED_{50}. Dose-response curves constructed for naive animals. The oxc temperature to 35°C was 80-fold greater t kg/hr. Time courses of recovery from a c change in metabolism occurring during c sion resulted in a decrease in the total nur low-affinity sites.

Marks, M. J., Artman, L. D. and *Collin*

Pharmacology Biochemistry & Behavior

Other support: Research Scientist Devel

From the Institute for Behavioral Genetics Center, University of Colorado, Boulder

INTRAVENOUS INJECTIONS OF NIC DISCRETE REDUCTIONS OF HYPOT LEVELS ASSOCIATED WITH INCRE PROLACTIN SECRETION

Previous studies have indicated that lamine (CA) nerve terminal systems are r tors. In view of the rapid onset of action seemed of interest at this time to analyze t and on neuroendocrine parameters such as

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Cancer Research Center.

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strains. Whereas nicotine
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in C3H mice. The doses of
C57BL mice but not in C3H
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3H mice exhibited enhanced
affinity and density of nico-
on for the differences among
or α-BTX binding sites did
wever, several differences in
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variation in response is not
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Marks, M. J., Burch, J. B. and *Collins, A. C.*

The Journal of Pharmacology and Experimental Therapeutics 226(1):291-302, 1983.

Other support: National Institutes of Health.

From the Institute for Behavioral Genetics and School of Pharmacy, University of Colorado, Boulder.

QUANTITATION OF TOLERANCE DEVELOPMENT AFTER CHRONIC OXOTREMORINE TREATMENT

An earlier study concerning the effects of chronic oxotremorine infusion provided clear evidence of the development of tolerance to oxotremorine. However, the method used to evaluate tolerance in that study did not permit a quantitative analysis of tolerance development. For the study presented here, a new procedure was developed to quantitate the tolerance which develops as mice are chronically infused with the muscarinic agonist. Cumulative dose-response curves were constructed for the effects of oxotremorine on body temperature and rotarod performance by administering sequential injections to individual animals. These dose-response curves compare favorably to those constructed by injecting individual animals with one of several doses. The sequential injection technique was used to assess the magnitude of tolerance development to oxotremorine. A linear relationship between oxotremorine infusion rate (dose) and magnitude of change of the ED_{50} value for impairment of rotarod performance was observed, with animals receiving an infusion rate of 1.0 mg/kg/hr showing a 24-fold increase in ED_{50}. Dose-response curves for tolerant animals were parallel to those constructed for naive animals. The oxotremorine dose required to decrease body temperature to 35°C was 80-fold greater than control in the group treated with 1.0 mg/kg/hr. Time courses of recovery from a challenge dose of oxotremorine suggest little change in metabolism occurring during chronic infusion. Chronic oxotremorine infusion resulted in a decrease in the total number of QNB binding sites in both high- and low-affinity sites.

Marks, M. J., Artman, L. D. and *Collins, A. C.*

Pharmacology Biochemistry & Behavior 19:103-113, 1983.

Other support: Research Scientist Development Award.

From the Institute for Behavioral Genetics, School of Pharmacy and Alcohol Research Center, University of Colorado, Boulder.

INTRAVENOUS INJECTIONS OF NICOTINE INDUCE VERY RAPID AND DISCRETE REDUCTIONS OF HYPOTHALAMIC CATECHOLAMINE LEVELS ASSOCIATED WITH INCREASES OF ACTH, VASOPRESSIN AND PROLACTIN SECRETION

Previous studies have indicated that the various types of hypothalamic catecholamine (CA) nerve terminal systems are regulated by nicotine-like cholinergic receptors. In view of the rapid onset of action of nicotine on the central nervous system, it seemed of interest at this time to analyze the action of nicotine on the hypothalamic CA and on neuroendocrine parameters such as prolactin, ACTH, vasopressin and corticos-

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

terone serum levels 2, 4 and 30 min. after an intravenous injection. Results presented here show that nicotine given i.v. can, within minutes, induce increases of ACTH, vasopressin and prolactin secretion in the male rat, giving further evidence for the existence of nicotine-like cholinergic receptors involved in the regulation of these hormones. These changes were associated with rapid reductions of noradrenaline (NA) levels in the subependymal layer of the median eminence, in the nuc. dorsomedialis hypothalami and in the anterior and posterior periventricular hypothalamic area as revealed by quantitative microfluorimetrical measurements of CA fluorescence. Intraindividual correlations indicate the involvement of an inhibitory noradrenergic mechanism in the subependymal layer of the median eminence in the regulation of ACTH secretion, the involvement of noradrenergic mechanisms in the posterior periventricular area in the regulation of prolactin secretion and the involvement of dopaminergic mechanisms in the medial palisade zone of the median eminence in the regulation of prolactin secretion. A rapid rise of prolactin secretion seems to be associated mainly with a reduction of NA levels in the posterior periventricular area indicating the existence of possible facilitatory noradrenergic mechanism in this region regulating prolactin secretion.

Andersson, K., Siegel, R., *Fuxe, K.*, and Eneroth, P.

Acta Physiologica Scandinavica 118(1):35-40, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet, Stockholm; Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden.

INVOLVEMENT OF CHOLINERGIC NICOTINE-LIKE RECEPTORS AS MODULATORS OF AMINE TURNOVER IN VARIOUS TYPES OF HYPOTHALAMIC DOPAMINE AND NORADRENALINE NERVE TERMINAL SYSTEMS AND OF PROLACTIN, LH, FSH AND TSH SECRETION IN THE CASTRATED MALE RAT

This paper provides evidence that repeated high subcutaneous doses of nicotine in the castrated male rat can reduce the amine levels in various hypothalamic dopamine (DA) and noradrenaline (NA) nerve terminal systems. Specifically, the effects of high repeated subcutaneous doses (4 × 2 mg/kg) of nicotine were evaluated on DA and NA levels and turnover in the long-term castrated male rat using catecholamine (CA) fluorescence histochemistry in combination with quantitative microfluorometry. The CA turnover was evaluated by studying the decline of the CA stores following tyrosine hydroxylase inhibition using α-methyltyrosine methyl ester (H 44/68). In the same experiments trunk blood was collected for the determination of serum prolactin, LH, FSH and TSH levels using standard radioimmunoassay procedures. The nicotine treatment produced a significant depletion of CA stores and an increase of CA turnover in DA and NA nerve terminals of the median eminence and in peri- and paraventricular NA systems. These effects were significantly counteracted by pretreatment with mecamylamine. Nicotine significantly reduced serum prolactin and TSH levels, and after H 44/68 it also reduced LH and FSH serum levels. Many of these actions were counteracted by mecamylamine pretreatment. Overall intraindividual correlations showed a significant correlation between reduced CA turnover in several hypothalamic areas and

increased serum LH and FSH leve
hypothalamic nucleus and increased
median eminence and increased ser

Andersson, K., *Fuxe, K.*, Eneroth,

Acta Physiologica Scandinavica 11(

Other support: Svenska Tobaks AI

From the Department of Histology,
partment of Obstetrics and Gynecc
and the Department of Human Phy

INTERACTIONS OF NICOTINE
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ADENOHYPOPHYSEAL HORM
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normal male rat. Results showed
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These effects were all antagonized
Pentobarbitone alone, however, di
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secretion. These results suggest,
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Andersson, K., *Fuxe, K.*, Eneroth

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Other support: Svenska Tobaks A

From the Department of Histology
the Hormone Laboratory, Departm
pital, Stockholm, Sweden, and th
Modena, Modena, Italy.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

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Stockholm; Laboratories of
, Department of Neurology,
Hormone Laboratory, De-
pital, Stockholm, Sweden.

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increased serum LH and FSH levels, increased NA turnover in the paraventricular hypothalamic nucleus and increased serum TSH levels, and reduce DA turnover in the median eminence and increased serum LH levels.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Acta Physiologica Scandinavica 116:41-50, 1982.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet; Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden, and the Department of Human Physiology, University of Modena, Modena, Italy.

INTERACTIONS OF NICOTINE AND PENTOBARBITONE IN THE REGULATION OF TELENCEPHALIC AND HYPOTHALAMIC CATECHOLAMINE LEVELS AND TURNOVER AND OF ADENOHYPOPHYSEAL HORMONE SECRETION IN THE NORMAL MALE RAT

The way(s) that nicotine may interact with pentobarbitone, a sedative hypnotic drug, in the regulation of activity in the different central dopamine (DA) and noradrenic (NA) pathways was evaluated in this study. To do this, the effects of single intraperitoneal injections of nicotine (1 mg/kg) and pentobarbitone (30 mg/kg) alone or in combination were studied on catecholamine (CA) nerve terminal system of the hypothalamus and the forebrain and on the adenohypophyseal hormone secretion of the normal male rat. Results showed that nicotine produced discrete reductions of DA levels and increases of DA turnover in striatal and limbic areas of the forebrain and increases of amine turnover in different hypothalamic NA nerve terminal systems. These effects were all antagonized by simultaneous treatment with pentobarbitone. Pentobarbitone alone, however, did not modulate CA levels or turnover in the various parts of the hypothalamus and forebrain analyzed. On the other hand, pentobarbitone increased GH and prolactin secretion and in association with tyrosine hydroxylase inhibition markedly reduced corticosterone secretion. These effects were partly counteracted by nicotine in the case of GH and prolactin secretion. Furthermore, a positive interaction appears to exist between nicotine and pentobarbitone in their actions on LH secretion. These results suggest, therefore, that pentobarbitone can antagonize the actions of nicotine on CA levels and turnover in various CA nerve terminal systems of the brain leading to possible reductions in nicotine-induced arousal and positive reinforcement. The neuroendocrine actions of pentobarbitone do not seem to be greatly modulated through nicotinic cholinergic receptors.

Andersson, K., *Fuxe, K.*, Eneroth, P., Agnati, L. F., and Gustafsson, J.-A.

Naunyn-Schmiedeberg's Archives of Pharmacology, 321:287-291, 1982.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology and Medical Nutrition, Karolinska Institutet, and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden, and the Department of Human Physiology, University of Modena, Modena, Italy.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

IMMOBILIZATION STRESS-INDUCED CHANGES IN DISCRETE HYPOTHALAMIC CATECHOLAMINE LEVELS AND TURNOVER, THEIR MODULATION BY NICOTINE AND RELATIONSHIP TO NEUROENDOCRINE FUNCTION

Male Sprague-Dawley rats were used in this evaluation of immobilization stress–nicotine interactions on hypothalamic catecholamine (CA) systems, and on neuroendocrine function, as manifested by the secretion of vasopressin, ACTH, corticosterone (CS), prolactin (PRL), LH and FSH. Immobilization stress (1 h) induced discrete reductions in noradrenaline (NA) levels in the posterior periventricular hypothalamic region and in the paraventricular hypothalamic nucleus, and a decrease in dopamine (DA) turnover in the medial palisade zone (MPZ) of the median eminence, but failed to induce regional increases of hypothalamic NA turnover. Stress also stimulated the secretion of ACTH, CS and PRL, while vasopressin, LH and FSH serum levels were unaffected. The stress-induced reduction of DA turnover in MPZ may mediate the stress-induced increase of PRL secretion. Nicotine did not by itself significantly influence CA turnover in the various CA nerve terminal systems analyzed in the hypothalamus, but it reduced NA levels in the subependymal layer of the median eminence. These changes in NA levels in discrete hypothalamic regions may be correlated with the stress-induced secretion of ACTH, since nicotine treatment to some extent counteracted the stress-induced changes in CA activity and in ACTH, but not in PRL secretion at the 1 h time interval studied. However, a stress-induced reduction of DA turnover in MPZ may be involved in causing the stress-induced increase of PRL secretion.

Fuxe, K. et al.

Acta Physiologica Scandinavica 117:421-426, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet, Stockholm; the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden; Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; and the Department of Human Physiology and Endocrinology, University of Modena, Modena, Italy.

RAPID AND DISCRETE CHANGES IN HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS INDUCED BY AUDIOGENIC STRESS, AND THEIR MODULATION BY NICOTINE–RELATIONSHIP TO NEUROENDOCRINE FUNCTION

The time course of audiogenic stress-induced changes in discrete hypothalamic dopamine (DA) and noradrenaline (NA) nerve terminal systems was studied using quantitative microfluorometric evaluations of catecholamine stores and radioimmunoassays for the determination of serum hormone levels, and was correlated with simultaneous stress effects upon the secretion of ACTH, corticosterone (CS), prolactin (PRL), and vasopressin. Finally, the effects of nicotine upon these parameters were evaluated alone or in association with stress. Audiogenic stress and nicotine induced very rapid and discrete decreases in NA levels in the subependymal layer (SEL), in the

parvocellular part of nuc. paraventricular periventricular hypothalamic systems, (P utes following the onset of treatment. Ir served after nicotine treatment but could r changes in catecholamine levels. These nicotine- and stress-induced increases c secretion found in these experiments. Stre vasopressin secretion induced by nicoti which stress combined with smoking can arterial blood pressure and finally to sust

Siegel, R. A., Andersson, K., *Fuxe, K.*, F

European Journal of Pharmacology 91(1

Other support: Svenska Tobaks AB, St

From the Laboratories of Experimental F Department of Neurology, Hadassah Un ment of Histology, Karolinska Institutet, Obstetrics and Gynecology, Karolinska of Human Physiology and Endocrinolog Research Laboratories, Astra Läkemede

EFFECTS OF PRENATAL ADMINIST ACID POOLS, PROTEIN METABOLI BRAIN

The material presented here indicate function, and shows that the processes newborn rat brain are affected by nicot effects of nicotine on brain protein met binding site in newborn animals expose synthesis rates measured *in vivo* were l Protein degradation rates measured *in vi* 13%. The effect was specific for L-(−)ni nicotinamide had no effect on degradat mainly nonessential amino acids and ar tion, were changed somewhat; an incr (21%), serine (35%) and glycine (35%) in the offspring of nicotine treated anim decrease in protein metabolism. Nicotin of animals exposed to nicotine during treatment of adult rats with nicotine, i binding site changed with age.

Sershen, H., Reith, M. E. A., Banay-S

Neurochemical Research 7(12):1515-152

From the Center for Neurochemistry, New York.

Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

parvocellular part of nuc. paraventricularis hypothalamic (PA FP) and in the posterior periventricular hypothalamic systems, (PV II); the decreases were apparent two minutes following the onset of treatment. Increases of arterial blood pressure were observed after nicotine treatment but could not have been a major factor in producing the changes in catecholamine levels. These changes in NA levels may be related to the nicotine- and stress-induced increases of ACTH (SEL and PA FP) and prolactin secretion found in these experiments. Stress enhanced the rapid but variable increase in vasopressin secretion induced by nicotine, suggesting one possible mechanism by which stress combined with smoking can contribute to the development of increased arterial blood pressure and finally to sustained hypertension.

Siegel, R. A., Andersson, K., *Fuxe, K.*, Eneroth, P., Lindbom, L-O, and Agnati, L. F.

European Journal of Pharmacology 91(1):49-56, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; Department of Histology, Karolinska Institutet, and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden; Departments of Human Physiology and Endocrinology, University of Modena, Modena, Italy; and Research Laboratories, Astra Läkemedel AB, Södertälje, Sweden.

EFFECTS OF PRENATAL ADMINISTRATION OF NICOTINE ON AMINO ACID POOLS, PROTEIN METABOLISM, AND NICOTINE BINDING IN THE BRAIN

The material presented here indicates that nicotine has diverse influences on brain function, and shows that the processes of protein degradation and synthesis in fetal newborn rat brain are affected by nicotine. Specifically, this study investigated the effects of nicotine on brain protein metabolism and on the properties of the nicotine binding site in newborn animals exposed to nicotine during gestation. Brain protein synthesis rates measured *in vivo* were lower by 18% in newborn of treated animals. Protein degradation rates measured *in vitro* in the presence of nicotine were lower by 13%. The effect was specific for L-(−)nicotine since D-(+)nicotine, nicotinic acid, or nicotinamide had no effect on degradation rates. Newborn brain amino acid levels, mainly nonessential amino acids and amino acids of putative neurotransmitter function, were changed somewhat; an increase in the level of taurine (13%), threonine (21%), serine (35%) and glycine (35%) and a decrease in lysine (14%) were observed in the offspring of nicotine treated animals. These changes could not account for the decrease in protein metabolism. Nicotine binding was higher by 25% in the offspring of animals exposed to nicotine during gestation. No such increase was found after treatment of adult rats with nicotine, indicating that the properties of the nicotine binding site changed with age.

Sershen, H., Reith, M. E. A., Banay-Schwartz, M., and *Lajtha, A.*

Neurochemical Research 7(12):1515-1522, 1982.

From the Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

V. *Pharmacology and Biochemistry*

DIFFERENTIAL SCANNING CALORIMETRY OF α_2-MACROGLOBULIN AND α_2-MACROGLOBULIN-PROTEINASE COMPLEXES

α_2-macroglobulin (α_2M), a major component of serum, appears to play a role in regulating the activity and concentration of endoproteinases since it has the capability of sequestering endoproteinases of all four major classes. In the study presented here, differential scanning calorimetry was shown to detect substantial structural alterations occurring on the association of proteinases with the serum glycoprotein α_2M. At pH 7.5, the thermally induced unfolding of the macroglobulin occurs at approximately 60° C with a transition enthalpy of 17J/g. Association of active thermolysin, trypsin and papain shifts the transition temperature to 77°C (transition enthalpy 5J/g), indicating that a substantial conformational change accompanies the binding event. The stoicheiometry of the thermolysin-α_2-macroglobulin association producing this change appears to be unity, implying the presence of cooperative subunit interactions in the mechanism of association. The calorimetric method provides a novel approach for the evaluation of conformational variants induced on protein-protein association or preexisting in the purified macroglobulin. As could be seen here, differential scanning calorimetry permits the ready detection of changes in the overall protein structure and is thus complementary to chemical and spectroscopic methods of analysis. In principle, the method can be employed to monitor behavior of α_2M in solutions more closely approximating the situation *in vivo* (*e.g.* plasma).

Chlebowski, J. F. and Williams, K.

Biochemical Journal 209:725-730, 1983.

Other support: National Institutes of Health.

From the Department of Biochemistry, Virginia Commonwealth University, Medical College of Virginia, Richmond.

THE SENSITIZATION OF THE TOXICITY OF SUPEROXIDE AND VITAMIN C BY COPPER AND IRON: A SITE SPECIFIC MECHANISM

In the study presented here, radiolysis of air-saturated solutions of sodium formate was used to generate superoxide. (This method yields superoxide as the predominant radical and seems to be the "cleanest" way to generate superoxide.) When superoxide's effect on a purified enzyme (penicillinase) and on bacteriophages T4 and T7 was studied in the absence of copper, it was found that the superoxide radicals were practically inert. However, when traces of Cu(II) were added, a dramatic enhancement of the damage with a dose modifying factor was observed. A similar copper-induced effect was noted when vitamin C instead of superoxide was added to air-saturated solutions of the enzyme acetylcholine esterase, bacteriophage T7 or the bacterium *Escherichia coli*. Experiments with isotopically labeled phages indicated that treating the phages with ascorbate and copper impaired their adsorption to the host, the injection of their DNA, as well as the expression of their genetic information within the host. Many of the observations discussed in this paper seem to be in agreement with a site specific or site directed mechanism. This mechanism, which may operate in many, though not necessarily all, biologic bound at or near sensitive sites.

Czapski, G. et al.

In: Cohen, G. and Greenwald, R. *tems*, New York: Elsevier Scienc *Aspects*, pp. 111-115.

Other support: Department of E Neuherberg, Federal Republic of C

From the Departments of Physical chemistry, the Hebrew University.

INACTIVATION OF T-EVEN AI SUPEROXIDE OR VITAMIN C

In this comparative study, the damage caused to various phages l difference was found in the respons bacteriophages were highly sensitiz the T-even phages appeared far mo addition of 10^{-7}M copper resulted i fying Factor >80. Lambda phag similar response. Polyethyleneglyc age mediated by superoxide and co the phages were irradiated in the ab in the sensitivities of the T-even a radiolytically formed O_2^- radicals c treatment with copper and vitami appears likely that the mechanism of copper to target biomolecules, radicals or vitamin C.

Aronovitch, J., Samuni, A. and C

In: Cohen, G. and Greenwald, R. *tems*, New York: Elsevier Scien *Aspects*, pp. 380-382.

Other support: Department of E Neuherberg, Federal Republic of

From the Departments of Molecu University, Jerusalem, Israel.

α-BUNGAROTOXIN LABELIN LOCALIZATION AT THE MAU HATCHETFISH

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

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hich may operate in many

though not necessarily all, biological systems, assumes that copper and iron ions are bound at or near sensitive sites.

Czapski, G. et al.

In: Cohen, G. and Greenwald, R. A. (eds.): *Oxy Radicals and their Scavenger Systems*, New York: Elsevier Science Publishing Co., Inc., 1983, vol. I: *Molecular Aspects*, pp. 111-115.

Other support: Department of Energy and Gesellschaft für strahlen Forschung, Neuherberg, Federal Republic of Germany.

From the Departments of Physical Chemistry, Molecular Biology and Cellular Biochemistry, the Hebrew University, Jerusalem, Israel.

INACTIVATION OF T-EVEN AND T-ODD COLIPHAGES INDUCED BY SUPEROXIDE OR VITAMIN C AND SENSITIZED BY COPPER

In this comparative study, the sensitizing effect of copper (II) ions on the radiation damage caused to various phages by superoxide radicals was noted, and a surprising difference was found in the response of T4 and T2 as compared to that of T7 phages: T7 bacteriophages were highly sensitized by copper towards superoxide radicals, whereas the T-even phages appeared far more resistant to this copper effect. With T7 phages the addition of 10^{-7}M copper resulted in a strikingly large sensitization with a Dose Modifying Factor >80. Lambda phages, which resemble the T-odd series, exhibited a similar response. Polyethyleneglycol did not provide any protection against the damage mediated by superoxide and copper. This is in contrast to the effect observed when the phages were irradiated in the absence of copper. In addition, the marked difference in the sensitivities of the T-even and T-odd phages to the copper-mediated effect of radiolytically formed O_2^- radicals closely paralleled the response of these phages to treatment with copper and vitamin C. Overall, based on these and other studies, it appears likely that the mechanism of sensitization by copper ions involves the binding of copper to target biomolecules, followed by their reduction by either superoxide radicals or vitamin C.

Aronovitch, J., Samuni, A. and *Czapski, G.*

In: Cohen, G. and Greenwald, R. A. (eds.): *Oxy Radicals and their Scavenger Systems*, New York: Elsevier Science Publishing Co., Inc., 1983, vol. I: *Molecular Aspects*, pp. 380-382.

Other support: Department of Energy and Gesellschaft für Strahlen Forschung, Neuherberg, Federal Republic of Germany.

From the Departments of Molecular Biology and Physical Chemistry, the Hebrew University, Jerusalem, Israel.

α-BUNGAROTOXIN LABELING AND ACETYLCHOLINESTERASE LOCALIZATION AT THE MAUTHNER FIBER GIANT SYNAPSE IN THE HATCHETFISH

In the medulla of the South American hatchetfish, the Mauthner fiber forms large chemical synapses on a number of large myelinated axons termed giant fibers. These axons mediate excitation of pectoral fin motoneurons bilaterally and in this fish bilat-

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

eral pectoral fin adduction is an important component of the Mauthner fiber-mediated escape reflex. For the study presented here, autoradiographic and histochemical techniques have been used to help characterize the pharmacology of transmission at the Mauthner fiber giant synapse of the hatchetfish. [^{125}I]α-Bungarotoxin was applied to hatchetfish medullae and a standard autoradiographic procedure was carried out on 3- to 4-μm sections of glutaraldehyde-fixed tissue. All Mauthner fiber giant synapses, as identified by light microscopic criteria, had closely associated silver grains. Labeling was blocked by *d*-tubocurarine. Glutaraldehyde-fixed slices of hatchetfish medulla were stained histochemically for acetylcholinesterase; all giant synapses that could be identified in the light microscope showed heavy deposits of reaction product. Staining was blocked by diisopropylfluorophosphate, which inhibits both pseudocholinesterase and acetylcholinesterase, but was not blocked by tetraisopropylpyrophosphoramide, a specific pseudocholinesterase inhibitor. This evidence strongly supports the suggestion that the Mauthner fiber synapse is nicotinic cholinergic.

Day, J. W., Hall, D. H., *Hall, L. M.*, and Bennett, M. V. L.

The Journal of Neuroscience 3(2):272-279, 1983.

Other support: National Science Foundation and the National Institutes of Health.

From the Division of Cellular Neurobiology, Department of Neuroscience, Albert Einstein College of Medicine, the Bronx, NY.

RECENT DEVELOPMENTS IN INHALATION TOXICOLOGY: EVALUATION OF SELECTED SHORT-TERM ENDPOINTS FOLLOWING 'NOSE-ONLY' EXPOSURE OF RODENTS

Inhalation toxicology has routinely employed traditional inhalation chamber exposures, which can result in a distribution pattern where only 10-30% of the particulates are deposited in the lungs of the exposed animals. A major portion of the material is deposited on the hides of the animals and as a result of preening, 60-80% of the material is eventually found in the gastrointestinal tract. A need for alternative exposure systems led to the development of a "nose-only" exposure system where the animal is restrained and the nose of each animal protrudes into an exposure chamber. Under such conditions, the dose to the lung can be optimized, controlled and documented. Employing a "nose-only" inhalation system and using one particular environmental aerosol, cigarette smoke, a battery of short-term endpoints was applied to assess the biological activity of the material. Three assays are discussed in this paper: a pharmacokinetics assay, a cellular proliferation assay and a cytogenetics assay. These assays demonstrated that exposure to cigarette smoke could result in certain smoke components (*e.g.*, dotriacontane) exerting effects directly in the lung, as reflected by the cellular proliferation assay in lung tissue, and other chemicals (*e.g.*, catechol) exerting effects systemically, as reflected by the cytogenetics assay in bone marrow.

Henry, C. J. *et al. (Microbiological Associates, Inc.)*

Other support: In: Homburger, F. (ed.): *Safety Evaluation and Regulation of Chemicals*, Basel: S. Karger, 1983, pp. 233-242.

From the Division of Toxicology and Oncology, Microbiological Associates, Inc., Bethesda, MD.

SEROTONIN-INDUCED DISRUPTIC
I. SERUM PROGESTERONE, IMPL/
AND INTRAUTERINE pO$_2$

The experiments described in this p disruption of implantation in the rat is as and/or uterine vascular function. The serotonin, administered on the day after nates pregnancy in the rat. Consequent terone levels, implantation site blood flc to see whether the disruption of implant uterine vascular function. Animals rece saline (C: control) or serotonin (S: 20 mg alter the number of blastocysts implant resorption. Progesterone levels in sero controls at 6 hrs. postinjection or on Da blood flow was reduced at 30 min. and injection. A prompt and sustained redu nied the reduced uterine perfusion. Thu luteum function but is associated with blood flow and intraluminal oxygen ava tion in the rat are compared with those

Mitchell, J. A. and Hammer, R. E.

Biology of Reproduction 28:830-835, 1

From the Department of Anatomy, W Detroit.

SEROTONIN-INDUCED DISRUPTIC
II. SUPPRESSION OF DECIDUALIZ

This study was undertaken to dete disrupt implantation in the rat, also sup of serotonin on decidualization, luteal f pseudopregnant rats; effects on serum p tation were determined in pregnant ani capable of disrupting implantation in p pseudopregnant animals. Further, the achieved by administration of serotonin serotonin-treated pseudopregnant ra pregnancy was not altered, and an injec suppress plasma progesterone levels ir suppression of decidualization is not a Rather, impaired decidualization proba uterine blood flow and possibly sustaine tion. The effects of serotonin on decidu

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

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Bungarotoxin was applied to
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I. V. L.

National Institutes of Health.

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XICOLOGY: EVALUATION
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SEROTONIN-INDUCED DISRUPTION OF IMPLANTATION IN THE RAT: I. SERUM PROGESTERONE, IMPLANTATION SITE BLOOD FLOW, AND INTRAUTERINE pO_2

The experiments described in this paper were undertaken to determine if serotonin disruption of implantation in the rat is associated with alterations in progesterone levels and/or uterine vascular function. The results of the first experiment showed that serotonin, administered on the day after the initiation of implantation, promptly terminates pregnancy in the rat. Consequently, the effects of serotonin on serum progesterone levels, implantation site blood flow and intrauterine O_2 tension were determined to see whether the disruption of implantation is related to altered corpus luteum and/or uterine vascular function. Animals received a subcutaneous injection of physiological saline (C: control) or serotonin (S: 20 mg/kg) on Day 5 of pregnancy. Serotonin did not alter the number of blastocysts implanting but did cause subsequent implantation site resorption. Progesterone levels in serotonin-treated rats did not differ from those of controls at 6 hrs. postinjection or on Days 6 through 10 of pregnancy. Implantation site blood flow was reduced at 30 min. and remained suppressed at 2 hrs. after serotonin injection. A prompt and sustained reduction in intrauterine oxygen tension accompanied the reduced uterine perfusion. Thus, disruption is not a result of impaired corpus luteum function but is associated with marked and protracted reductions in uterine blood flow and intraluminal oxygen availability. The effects of serotonin on implantation in the rat are compared with those of nicotine.

Mitchell, J. A. and Hammer, R. E.

Biology of Reproduction 28:830-835, 1983.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

SEROTONIN-INDUCED DISRUPTION OF IMPLANTATION IN THE RAT: II. SUPPRESSION OF DECIDUALIZATION

This study was undertaken to determine whether serotonin, at a dose known to disrupt implantation in the rat, also suppresses decidualization. To do this, the effects of serotonin on decidualization, luteal function or uterine blood flow were assessed in pseudopregnant rats; effects on serum progesterone levels and maintenance of implantation were determined in pregnant animals. Results indicate that doses of serotonin capable of disrupting implantation in pregnant rats also suppress decidualization in pseudopregnant animals. Further, the impairment of both phenomena is maximally achieved by administration of serotonin on Day 5. It is inferred that progesterone levels in serotonin-treated pseudopregnant rats are normal since the duration of pseudopregnancy was not altered, and an injection of an equivalent dose of serotonin did not suppress plasma progesterone levels in pregnant rats. Therefore, serotonin-induced suppression of decidualization is not a consequence of compromised luteal activity. Rather, impaired decidualization probably results from ischemia initiated by reduced uterine blood flow and possibly sustained and intensified by subsequent emboli formation. The effects of serotonin on decidualization are compared with those of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

Mitchell, J. A., Hammer, R. E. and Goldman, H.

Biology of Reproduction 29:151-156, 1983.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

CONCOMITANT REDUCTION IN UTERINE BLOOD FLOW AND INTRAUTERINE OXYGEN TENSION IN THE RAT FOLLOWING NICOTINE ADMINISTRATION

This study represents an attempt to determine the relationship between uterine blood flow and the availability of oxygen within the uterus and the effects of nicotine on such vascular-dependent phenomena as blood flow and capillary permeability at the implantation site. Pregnant Sprague-Dawley rats received an injection at 1200 h, day 5; pseudopregnant animals were injected between 1300 and 1400 h on day 4. Controls received a sc injection of an equivalent volume of saline. Measurements were then made of intrauterine oxygen, blood flow in whole uteri and at implantation sites, and of endometrical vascular permeability. Results show that nicotine suppresses whole uterine blood flow and luminal oxygen concentration, decreases perfusion at the implantation site and retards growth of the nidus. Whereas prior to implantation the well being of the free-living conceptus is dependent on the diffusion of blood-bones metabolic substrates from the endometrial vasculature to the uterine lumen; following nidation, maintenance of optimal conditions for continuation of pregnancy is dependent on metabolic exchange between the embryo and its implantation chamber. Nicotine, being capable of altering uterine vascular function both before and after uterine-blastocyst contact, exerts diverse effects on the course of pregnancy in the rat. The decreased concentration of oxygen and other metabolic substrates consequent to nicotine-induced reduction in uterine blood flow probably contributes to such preimplantation phenomena as reduced conceptus growth, delayed implantation and suppressed decidualization. Whereas once implantation is underway, the alkaloid retards embryo and/or nidus growth as indicated by reduced implantation site weight.

Mitchell, J. A., Hammer, R. E. and Goldman, H.

In: Bicher, H. I. and Bruley, D. F. (eds): *Oxygen Transport to Tissue–IV*, New York: Plenum Press Corporation, 1983, pp. 231-241.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

EFFECT OF GTP ANALOGUES ON PURIFIED SOLUBLE GUANYLATE CYCLASE

Although soluble guanylate cyclase from several mammalian sources has been purified recently, no information is available as of now on the stoichiometry of substrate binding or on the requirements of the nucleotide binding site. In this attempt to gain insight into these questions, as well as to understand more clearly the complex kinetic characteristics of guanylate cyclase, the effects of various nucleotide analogues on the enzyme were examined. Three GTP analogues, 5-guanylylimidodiphosphate (GMP-P(NH)P), guanylyl-(β,γ-methylene) diphosphate (GMP-(CH_2)P), and guanosine 5′-*O*-(3-thiotriphosphate) (GT
cyclase. GTPγS suported cyclic G
Mn^{2+}-GTP and Mg^{2+}-GTP, respecti
cyclic GMP formation at 10-20% c
analogues were found to have mult
sine triphosphate, and the 2′3′-dialc
for the enzyme; however, they we
logues could be useful tools for the s
they may also help elucidate the eff
cyclase regulation.

Brandwein, H. J., Lewicki, J. A.,

The Journal of Biological Chemist

Other support: National Institutes

From the Departments of Medicine
Veterans Administration Medical C

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within medium spiny neurons of
neocortex. Cerebellar guanylate cy
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Ariano, M. A., Lewicki, J. A., Br

Proceedings of the National Acad
79:1316-1320, 1982.

Other support: Biomedical Resea
College of Medicine, and the Nati

From the Department of Anatomy
of Medicine, Burlington, and the D
ford University, Palo Alto Veteran

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

Wayne State University

LOW AND
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ıtionship between uterine
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ı injection at 1200 h, day 5;
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various nucleotide analogues
5-guanylylimidodiphosphate
(GMP-(CH_2)P), and guano-

sine 5′-*O*-(3-thiotriphosphate) (GTPγS) were found to be substrates for guanylate cyclase. GTPγS suported cyclic GMP formation at 20 or 75% of the rate seen with Mn^{2+}-GTP and Mg^{2+}-GTP, respectively. GMP-P(NH)P and GMP-P(CH_2)P supported cyclic GMP formation at 10-20% of the GTP rate with either cation cofactor. These analogues were found to have multiple K_m values. Guanosine tetraphosphate, adenosine triphosphate, and the 2′3′-dialdehyde derivative of GTP were not good substrates for the enzyme; however, they were potent competitive inhibitors. These GTP analogues could be useful tools for the study of GTP binding sites on guanylate cyclase and they may also help elucidate the effects of free radicals and other agents on guanylate cyclase regulation.

Brandwein, H. J., Lewicki, J. A., Waldman, S. A., and *Murad, F.*

The Journal of Biological Chemistry 257(3):1309-1311, 1982.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

IMMUNOHISTOCHEMICAL LOCALIZATION OF GUANYLATE CYCLASE WITHIN NEURONS OF RAT BRAIN

The immunohistochemical localization of guanylate cyclase has been described in three central nervous system areas (rat neocortex, caudate-putamen and cerebellum) by using four different monoclonal antibodies directed against the soluble form of rat lung enzyme. Immunofluorescence could be seen within somata and proximal dendrites of neurons in these regions. A nuclear immunofluorescence reaction to guanylate cyclase was characteristically absent. The staining pattern for guanylate cyclase was coincident with previously described localizations of cyclic GMP immunofluorescence within medium spiny neurons of the caudate-putamen and pyramidal cells of the neocortex. Cerebellar guanylate cyclase immunoreactivity was primarily confined to Purkinje cells and their primary dendrites, similar to the pattern reported for cyclic GMP-dependent protein kinase localization. Guanylate cyclase immunofluorescence was abolished when the monoclonal antibodies were exposed to purified enzyme prior to incubation of the tissue slices or when control antibody was substituted for the primary antibody. Immunohistochemical localization of cyclic AMP in these same tissues was readily distinguished from that of guanylate cyclase or cyclic GMP, showing uniform fluorescence throughout the cell bodies of neurons and glial elements. The predominantly neuronal location of cyclic GMP, cyclic GMP phosphodiesterase, cyclic GMP-dependent protein kinase, and guanylate cyclase discussed here suggests a role for cyclic GMP in neurotransmission.

Ariano, M. A., Lewicki, J. A., Brandwein, H. J., and *Murad, F.*

Proceedings of the National Academy of Sciences of the United States of America 79:1316-1320, 1982.

Other support: Biomedical Research Support Grant to the University of Vermont College of Medicine, and the National Science Foundation.

From the Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, and the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

1002318983

Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

PROPERTIES OF PURIFIED SOLUBLE GUANYLATE CYCLASE ACTIVATED BY NITRIC OXIDE AND SODIUM NITROPRUSSIDE

For the experiments presented here, highly purified rat lung soluble guanylate cyclase was activated with nitric oxide or sodium nitroprusside and the degree of activation varied with incubation conditions. With Mg^{2+} as the cation cofactor, about 2- to 8-fold activation was observed with nitric oxide or sodium nitroprusside alone. Markedly enhanced activation (20-40 fold) was observed when 1μM hemin was added to the enzyme prior to exposure to the activating agent. Activation with hemin and sodium nitroprusside was prevented in a dose-dependent manner by sodium cyanide. The level of activation was increased by the addition of 1 mM dithiothreitol, but unlike hemin which had no effect on basal enzyme activity, dithiothreitol led to a considerable increase in basal activity. In another study, of the reversibility of the activation of guanylate cyclase by nitric oxide and sodium nitroprusside, it was seen that activated guanylate cyclase decayed to basal activity within one hour at 2°C and the enzyme could be reactivated upon re-exposure to nitroprusside or nitric oxide. Under basal conditions, Michaelis-Menten kinetics were observed, with a K_m for GTP of 140 μM with Mg^{2+} cofactor. Following activation with nitroprusside or nitric oxide, curvilinear Eadie-Hofsteen transformations of kinetic data were observed, with K_m's of 22 μM and 100 μM for Mg-GTP. Similar curvilinear Eadie-Hofstee transformations were observed with Mn^{2+} as the cation cofactor. These and other data suggest that multiple GTP catalytic sites are present in activated guanylate cyclase or, alternatively, multiple populations of enzyme exist.

Lewicki, J. A., Brandwein, H. J., Mittal, C. K., Arnold, W. P., and *Murad, F.*

Journal of Cyclic Nucleotide Research 8(1):17-25, 1982.

Other support: National Institutes of Health and National Research Service Awards.

From the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA, and the Department of Anesthesiology, University of Virginia, Charlottesville.

SODIUM NITROPRUSSIDE-INDUCED PROTEIN PHOSPHORYLATION IN INTACT RAT AORTA IS MIMICKED BY 8-BROMO CYCLIC GMP

This pharmacological study with intact rat thoracic aorta demonstrates that nitroprusside can alter the incorporation of ^{32}P into proteins from soluble and particulate fractions of the aorta. Furthermore, the pattern of altered protein phosphorylation evaluated with two-dimensional gel electrophoresis was mimicked by 8-bromo cyclic GMP. Approximately 100- and 10-fold greater concentrations of 8-bromo cyclic AMP and dibutyryl cyclic AMP, respectively, were required in order to increase ^{32}P incorporation into certain proteins whose phosphorylation was altered by 8-bromo cyclic GMP or nitroprusside. Isoproterenol also increased ^{32}P incorporation into some proteins: the incorporation of ^{32}P into some proteins was preferentially increased by dibutyryl cyclic AMP. Overall, the pattern of ^{32}P incorporation induced by a relatively high concentration of isoproterenol 0.1 mM was similar but not identical to that seen with 0.5 mM dibutyryl cyclic AMP. This study indicates that the incorporation of ^{32}P into endogenous proteins of intact rat aorta can be regulated by nitroprusside. These effects can be mimicked by cyclic GMP analogues and only partially by cyclic AMP analogues or isoproterenol. Presumably, these effects of nitroprusside are mediated through a cyclic

GMP-dependent process (protein ki
relaxant properties of nitroprusside

Rapoport, R. M., Draznin, M. B. a

Proceedings of the National Acad
79:6470-6474, 1982.

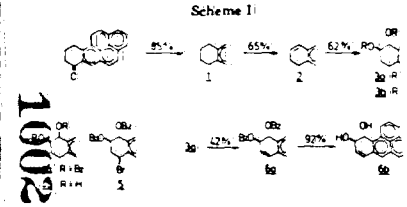
Other support: National Institutes o
Research Service Award.

From the Department of Medicine a
Veterans Administration Medical C

EFFICIENT SYNTHESIS OF NO
POLYCYCLIC AROMATIC HYD
CATECHOLS

Non-K-region dihydrodiols of
important role in the metabolism o
epoxides, some of which are consi
PAH. The two currently used meth
introduction of the *trans*-diol struc
reaction or the reduction of a suita
cumbersome and not too efficient. I
tion of non-K-region *o*-quinones w
only when the olefinic bond was fi
found that non-K-region *o*-quinone
sodium borohydride if the reaction
summarizes the results obtained wit
reduced with sodium borohydride.
chols, e.g., 3a, and their diacetates,
e.g., 1a, all three compounds being
reduced with sodium borohydride i

Scheme I



The results presented in this paper
atechols can be efficiently and co
borohydride, in the presence of oxyg
dihydrodiols has the additional adva
workup and thus competes favorabl

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

GMP-dependent process (protein kinase or phosphatase) which may play a role in the relaxant properties of nitroprusside and cyclic GMP.

Rapoport, R. M., Draznin, M. B. and *Murad, F.*

Proceedings of the National Academy of Sciences of the United States of America 79:6470-6474, 1982.

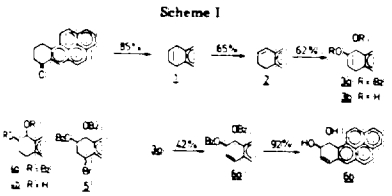
Other support: National Institutes of Health, Veterans Administration, and National Research Service Award.

From the Department of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

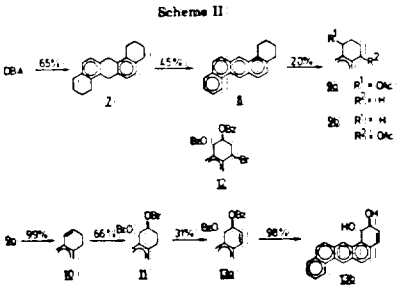
EFFICIENT SYNTHESIS OF NON-K-REGION *TRANS*-DIHYDRODIOLS OF POLYCYCLIC AROMATIC HYDROCARBONS FROM *O*-QUINONES AND CATECHOLS

Non-K-region dihydrodiols of polycyclic aromatic hydrocarbons (PAH) play an important role in the metabolism of PAH since they are precursors of dihydrodiol epoxides, some of which are considered to be ultimate carcinogenic metabolites of PAH. The two currently used methods for the synthesis of *trans*-dihydrodiols – the introduction of the *trans*-diol structure into a suitable dihydroarene by the Prévost reaction or the reduction of a suitable *o*-quinone with complex metal hydrides – are cumbersome and not too efficient. Following a different method, the efficient reduction of non-K-region *o*-quinones with sodium borohydride seemed possible before only when the olefinic bond was first converted to the dibromide. However, it was found that non-K-region *o*-quinones can be efficiently reduced to dihydrodiols with sodium borohydride if the reaction is performed in the presence of oxygen. Table I summarizes the results obtained with 1,2-dioxygenated derivatives of naphthalene are reduced with sodium borohydride. It should be emphasized that non-K-region catechols, e.g., 3a, and their diacetates, e.g., 5a, can conveniently replace the *o*-quinones, e.g., 1a, all three compounds being converted to the dihydrodiol, e.g., 4a, if they are reduced with sodium borohydride in the presence of oxygen.

Scheme I



Scheme II



The results presented in this paper demonstrate that non-K-region *o*-quinones and catechols can be efficiently and conveniently reduced to dihydrodiols with sodium borohydride, in the presence of oxygen. This method of preparing non-K-region *trans*-dihydrodiols has the additional advantage of a very simple experimental procedure and workup and thus competes favorably with the earlier methods of preparation.

10023189B5

Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

Platt, K. L. and *Oesch, F.*

The Journal of Organic Chemistry 48:265-268, 1983.

From the Institute of Pharmacology, Section on Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

FREE RADICAL PATHOLOGY: INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY PRODUCTS FROM THE REACTION OF NITROGEN DIOXIDE WITH HYDROGEN PEROXIDE AND THE ETIOLOGY OF EMPHYSEMA

This paper presents a study of the production of biologically damaging species from the reaction of nitrogen dioxide with hydrogen peroxide using the oxidative inactivation of human α-1-proteinase inhibitor as a model reaction. In this work, exposure of human α-1-proteinase inhibitor to 75 ppm nitrogen dioxide (NO_2) and 1 mM hydrogen peroxide (H_2O_2) resulted in a 65% loss in the anti-elastase activity of the protein after one hour. Exposure to either NO_2 or H_2O_2 alone had no effect. The inhibition was partially prevented when mannitol or superoxide dismutase was included during the exposure, implying that superoxide and the hydroxyl radical are formed in reactions between NO_2 and H_2O_2 and play a role in the inhibitory process. In conclusion, in this paper the authors have demonstrated the inactivation of the anti-elastase activity of human α-1-proteinase by species produced from the reaction of NO_2 with H_2O_2. The inhibition of this inactivation by mannitol and superoxide dismutase implies that these reactive species include superoxide and the hydroxyl radical, and may, upon extrapolation of the data, react directly with elastin and other lung tissue in direct damage-producing reactions.

Dooley, M. M. and *Pryor, W. A.*

Biochemical and Biophysical Research Communications 106(3):981-987, 1982.

Other support: National Institutes of Health and the National Science Foundation.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

ELECTRON-SPIN RESONANCE STUDY OF MAINSTREAM AND SIDESTREAM CIGARETTE SMOKE: NATURE OF THE FREE RADICALS IN GAS-PHASE SMOKE AND IN CIGARETTE TAR

In this chemical study, radicals in the gas phase of both mainstream and sidestream cigarette smoke have been monitored using electron-spin resonance (ESR) spin-trapping techniques with α-phenyl-N-*tert*-butyl nitrone (PBN) as the spin-trap. The principal radicals trapped in this way appear to be alkoxyl radicals. Mainstream and sidestream gas-phase smoke each has about the same concentration of radicals, about 1×10^{16} radicals per cigarette (or 5×10^{14} per puff). These radicals are reactive, yet they appear to be remarkably long-lived since they are still spin-trapped from gas-phase smoke after more than five min. The authors propose that a steady-state concentration of reactive radicals exists in gas phase cigarette smoke. They suggest that this steady state is produced by the slow oxidation of nitric oxide (present in high concentrations in smoke and relatively unreactive) to the more reactive nitrogen dioxide, followed by the reaction of nitrogen dioxide with reactive organic molecules in smoke (such as olefins and dienes). Preliminary experiments reported here support this hy-

pothesis. It is also shown in this pap
smoke contains persistent free radical
g values of 2.003. The tar radical c
organic solvents, and a variety of fra
solutions. Most of the radicals occur
leaf pigments. Treatment of alcoholic
of radicals that appear to be semiqui
phenolic and polyphenolic species in

Pryor, W. A., Prier, D. G. and Chur

Environmental Health Perspectives 4

Other support: National Institutes of

From the Department of Chemistry,

REACTIVE OXY-RADICALS FRO PHYSIOLOGICAL EFFECTS

Cigarette smoke contains high cc
in tar. In this paper, the nature of the
and it is suggested that radical-mediat
logical effects of smoke. Sections
RADICAL, THE GAS PHASE SMC
SMOKE, CELLULOSE "CIGARET
and ADDITIONAL RADICAL MEC
TEASE. In summary, it seems that al
pulmonary damage. Alkoxyl radicals
nary target molecules such as unsatu
proteins. Smoke also stimulates pulm
addition, NO_2 can react with the hy
radicals and/or superoxide. Finally, t
the tar radical) might cause the oxidat
smoke to carcinogenic species.

Pryor, W. A., et al.

In: Greenwald/Cohen (eds.): *Oxy Radi*
Medical Aspects, New York: Elsevier

Other support: National Science Fou
National Foundation for Cancer Rese

From the Department of Chemistry, L

ENKEPHALIN- AND SUBSTANCE MAMMALIAN SPERM AND ACCE

For the study presented here, enk
stance P were extracted from human, b
and accessory sex glands of the rat;
and the biological activity of peptides
higher levels of methionine enkephali

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

pothesis. It is also shown in this paper that tar from both mainstream and sidestream smoke contains persistent free radicals that exhibit broad, single-line ESR spectra with *g* values of 2.003. The tar radical can be extracted into *tert*-butylbenzene and other organic solvents, and a variety of fractionation procedures have been applied to these solutions. Most of the radicals occur in the fractions that contain the phenolic tobacco leaf pigments. Treatment of alcoholic solutions of tar with base generates a new group of radicals that appear to be semiquinone radicals derived from the oxidation of the phenolic and polyphenolic species in tar.

Pryor, W. A., Prier, D. G. and Church, D. F.

Environmental Health Perspectives 47:345-355, 1983.

Other support: National Institutes of Health.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

REACTIVE OXY-RADICALS FROM CIGARETTE SMOKE AND THEIR PHYSIOLOGICAL EFFECTS

Cigarette smoke contains high concentrations of radicals both in the gas phase and in tar. In this paper, the nature of the radicals in tar and in the gas phase are discussed and it is suggested that radical-mediated mechanisms account for some of the physiological effects of smoke. Sections of the paper are devoted to THE TAR FREE RADICAL, THE GAS PHASE SMOKE FREE RADICAL, NO_x CHEMISTRY IN SMOKE, CELLULOSE "CIGARETTE" AND EXCITED SPECIES IN SMOKE, and ADDITIONAL RADICAL MECHANISMS–DESTRUCTION OF ANTIPROTEASE. In summary, it seems that all of the radical-producing processes might cause pulmonary damage. Alkoxyl radicals in smoke could directly attack reactive pulmonary target molecules such as unsaturated lipids or sulfur-containing enzymes and proteins. Smoke also stimulates pulmonary macrophages to produce O_2^- and H_2O_2. In addition, NO_2 can react with the hydrogen peroxide produced to release hydroxyl radicals and/or superoxide. Finally, the radicals in smoke (peroxyl, alkoxyl, NO_2, or the tar radical) might cause the oxidation of the polycyclic aromatic hydrocarbons in smoke to carcinogenic species.

Pryor, W. A., et al.

In: Greenwald/Cohen (eds.): *Oxy Radicals and Their Scavenger Systems: Cellular and Medical Aspects*, New York: Elsevier, 1983, vol. II, pp. 185-192.

Other support: National Science Foundation, National Institutes of Health and the National Foundation for Cancer Research.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

ENKEPHALIN- AND SUBSTANCE P-LIKE IMMUNOREACTIVITIES OF MAMMALIAN SPERM AND ACCESSORY SEX GLANDS

For the study presented here, enkephalins and related opioid peptides, and Substance P were extracted from human, bull and rat spermatozoa, human seminal plasma and accessory sex glands of the rat; radioimmunoassays were run on these extracts and the biological activity of peptides was characterized. Results showed there were higher levels of methionine enkephalin and leucine enkephalin in seminal plasma than

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

in spermatozoa and higher levels of Substance P were present in sperm cells than in plasma. Specifically, the extracts of spermatozoa, seminal plasma and accessory sex glands produced the following responses: (1) Extracts containing very high immunoreactivities to opioid peptides compared to Substance P (*e.g.*, extract of human seminal plasma) inhibited chemical transmission. (2) Extracts containing very high immunoreactivities to Substance P compared to the opioid peptide (*e.g.*, caudal epididymis) facilitated transmission. (3) All other extracts caused biphasic responses. Overall, these results indicate methionine enkephalin and Substance P may play a regulatory role in acetylcholine-induced Ca^{2+} movements and, therefore, progressive sperm motility.

Sastry, B. V. R. et al.

Biochemical Pharmacology 31(21):3519-3522, 1982.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

CHANGES IN ENZYMES OF THE CHOLINERGIC SYSTEM AND ACETYLCHOLINE RELEASE IN THE CEREBRA OF AGING MALE FISCHER RATS

Since the functional decline of memory in the aging human brain has been partially attributed to defects in cholinergic transmission, it was decided to investigate various components of the cholinergic system in cerebra of Fischer 344 male rats, ages 3-33 months. Choline acetyltransferase (ChA), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) activities were determined in homogenates of the cerebra using specific radiometric assays. For measuring the release of acetylcholine (ACh), cerebral slices were incubated for one hour in Krebs buffer containing ^{3}H-choline chloride to label ACh formed *in situ*, washed, and transferred to a microbath for superfusion. ^{3}H-ACh released into the superfusate was determined. Results showed that the levels of ChA in the cerebra of 9- to 27-month-old rats were lower than those in 3-month-old rats. Only 1% of these rats survive to the age of 33 months, while in rats of this age there was no decrease in ChA levels. The survival of a small group of Fischer 344 rats up to 33 months and the maintenance of ChA activities in this group suggests the possibility of an interaction of age and genetic factors in the control of cortical cholinergic mechanisms. In addition, AChE decreased while BChE increased with advancing age. The rate of spontaneous release of ^{3}H-ACh decreased gradually by 63% from 3 to 33 months of age. The evoked release of ACh decreased by 50% in 33-month-old rats. Alterations in the levels of ChA, AChE (or BChE) and cholinergic receptors are not large enough to account for losses in cholinergic transmission in the cerebrum. The large drops in the rates of spontaneous or evoked release of ACh in the aging cerebrum indicate that the functional defect in the cholinergic transmission of the aging cerebrum is possibly due to a defective release mechanism of this transmitter.

Sastry, B. V. R. et al.

Pharmacology 26:61-72, 1983.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

DEPRESSION OF HUMAN SPERM ENZYMATIC METHYLATION

This report describes, for the firs
ance to motility of phospholipid N-m
In this work, enzymatic phospholipic
consequences of their inhibition on n
date here suggest that there are at least
first enzyme, PMT I, converted mem
phosphatidyl-N-methylethanolamine (
of 8.0. The second PMT converted PM
and PC. It had a K_m of 71 μM and a pH
protein carboxymethylase (PCM) and
lar levels of S-adenosylhomocysteine
mediated methylations, were increase
tone (L-HCT), and erythro-9-(2-hydr
adenosine deaminase, to human sperm
suspension was determined every hou
adenosine, L-HCT and EHNA, the
similar conditions, phospholipid meth
ments were also conducted in the prese
in the inhibition of phospholipid methy
homogenates had the following order
indicate that the PMT system and/or t
the regulation of human sperm motilit

Sastry, B. V. R. and Janson, V. E.

Biochemical Pharmacology 32(8):142

Other support: National Institute of (
National Institute of Aging.

From the Department of Pharmacolog
Nashville, TN.

REACTION OF PEPTIDE THIOBEN CHYMOTRYPSINLIKE ENZYMES

Several chymotrypsinlike serine p
turnover of various proteins, have re
include rat mast cell protease I, rat mas
enzyme, dog skin chymotrypsinlike e
vine chymotrypsin A. In this attempt t
these enzymes, kinetic constants as we
of the peptide substrates succinyl-phen
and succinyl-alanyl-alanyl-prolyl-phe

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

sent in sperm cells than in
plasma and accessory sex
aining very high immuno-
., extract of human seminal
aining very high immuno-
: (*e.g.*, caudal epididymis)
ic responses. Overall, these
nay play a regulatory role
progressive sperm motility.

ersity School of Medicine,

'STEM AND
AGING MALE FISCHER

human brain has been par-
was decided to investigate
Fischer 344 male rats, ages
inesterase (AChE), and bu-
homogenates of the cerebra
ase of acetylcholine (ACh),
uffer containing ^{3}H-choline
nsferred to a microbath for
letermined. Results showed
rats were lower than those in
of 33 months, while in rats of
l of a small group of Fischer
ivities in this group suggests
rs in the control of cortical
while BChE increased with
decreased gradually by 63%
h decreased by 50% in 33-
(or BChE) and cholinergic
olinergic transmission in the
evoked release of ACh in the
olinergic transmission of the
chanism of this transmitter.

iversity School of Medicine,

DEPRESSION OF HUMAN SPERM MOTILITY BY INHIBITION OF ENZYMATIC METHYLATION

This report describes, for the first time, the occurrence, properties and significance to motility of phospholipid N-methyltransferase (PMT) in human spermatozoa. In this work, enzymatic phospholipid methylation and carboxymethylation, and the consequences of their inhibition on motility, were studied using human sperm. The date here suggest that there are at least two PMT enzymes in human spermatozoa. The first enzyme, PMT I, converted membrane bound phosphatidylethanolamine (PE) to phosphatidyl-N-methylethanolamine (PME). It had a K_m of 4.0 μM and a pH optimum of 8.0. The second PMT converted PME to phosphatidyl-N,N-dimethylethanolamine and PC. It had a K_m of 71 μM and a pH optimum of 10.0. Spermatozoa also contained protein carboxymethylase (PCM) and methyl acceptor protein (MAP). The intracellular levels of S-adenosylhomocysteine (SAH), an inhibitor of S-adenosylmethionine-mediated methylations, were increased by adding adenosine, L-homocysteine thiolactone (L-HCT), and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), an inhibitor of adenosine deaminase, to human sperm ejaculates. The motility index of each sperm suspension was determined every hour for four hrs. In the presence of the mixture of adenosine, L-HCT and EHNA, the motility index was depressed by 57%. Under similar conditions, phospholipid methylation was depressed by 48%. Similar experiments were also conducted in the presence of 3-deazaadenosine. The potencies of SAH in the inhibition of phospholipid methylation and protein carboxymethylation in sperm homogenates had the following order: PMT I>PCM>PMT II. These observations indicate that the PMT system and/or the PCM-MAP system play a significant role in the regulation of human sperm motility.

Sastry, B. V. R. and Janson, V. E.

Biochemical Pharmacology 32(8):1423-1432, 1983.

Other support: National Institute of Child Health and Human Development and the National Institute of Aging.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REACTION OF PEPTIDE THIOBENZYL ESTERS WITH MAMMALIAN CHYMOTRYPSINLIKE ENZYMES: A SENSITIVE ASSAY METHOD

Several chymotrypsinlike serine proteases, which are thought to play a role in the turnover of various proteins, have recently been isolated and characterized. These include rat mast cell protease I, rat mast cell protease II, human skin chymotrypsinlike enzyme, dog skin chymotrypsinlike enzyme, human leukocyte cathepsin G, and bovine chymotrypsin A. In this attempt to develop a sensitive method for the detection of these enzymes, kinetic constants as well as the maximum sensitivity for the hydrolysis of the peptide substrates succinyl-phenyl-alanyl-leucyl-phenylalanine thiobenzyl ester and succinyl-alanyl-alanyl-prolyl-phenylalanine thiobenzyl ester were determined.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

Hydrolysis rates were followed spectrophotometrically at 324 nm by the formation of 4-thiopyridone, the product of the reaction between benzylthiol, released during hydrolysis of the peptide thiobenzyl esters, and 4,4′-dithiodipyridine present in the assay mixture. Peptide thiobenzyl ester substrates were shown to be very sensitive substrates, predominantly because of the large extinction coefficient of 4-thiopyridine and the high k_{cat}/K_m values for these compounds. Overall, the assays reported here are more sensitive than other methods previously reported and it seems that these thioester substrates could be extremely useful in future studies of the physiological role of these mammalian chymotrypsinlike enzymes.

Harper, J. W., Ramirez, G. and Powers, J. C. *(Travis, J.)*

Analytical Biochemistry 118:382-387, 1981.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

EFFECT OF CIGARETTE SMOKING ON BASAL AND CARBON DIOXIDE STIMULATED CEREBRAL BLOOD FLOW IN MAN

This study was undertaken to determine whether smoking is paralleled by a decreased, increased or unaffected cerebral blood flow (CBF). Healthy habitual cigarette smokers, ten men and three women, aged 21-48 years, were tested for the effect of smoking on CBF and on the cerebral hyperemia induced by CO_2 breathing. CBF was measured with the N_2O-wash-in technique in the basal state and during inhalation of 5% CO_2, before and after smoking of two commercial filter tipped cigarettes. In parallel, the (arterial-jugular venous) difference in O_2 content, arterial and jugular venous $_pCO_2$, pulmonary ventilation, heart rate and systemic blood pressure were followed. Results showed that during smoking there was a 10-15 mm Hg increase in systemic blood pressure and a parallel elevation of heart rate (+20 beats/min). CBF increased by about 25%, and cerebral vascular resistance fell about 15%. The cerebral metabolic rate of oxygen ($CMRO_2$) was elevated by about 30% above control. Inhalation of 5% CO_2 by itself markedly increased CBF and decreased cerebral vascular resistance, while leaving $CMRO_2$ unaffected. Cigarette smoking did not significantly change either of these effects of CO_2 breathing. Overall, from these data it is concluded that cigarette smoking elevates systemic blood pressure and decreases cerebral vascular resistance, and thereby augments basal CBF. This flow-promoting effect of smoking is probably due to an increased cerebral consumption of oxygen. Furthermore, the data demonstrate that smoking does not interfere with the cerebral vascular response to increased arterial $_pCO_2$.

Wennmalm, Å.

Clinical Physiology 2(6):529-535, 1982.

Other support: The Swedish Tobacco Company.

From the Department of Clinical Physiology, Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

NICOTINE INHIBITS VASCULAI THROMBOXANE FORMATION

The metabolism of arachidonic involves formation of a number of p taglandins (PG), thromboxane (Tx), paper, the metabolism of AA in two lium – is discussed with special ref metabolism. The effect of nicotine c from human platelets, while the effe was studied in rings of rabbit aorta presence of nicotine or indometha [^{14}C]arachidonic acid to [^{14}C]thromb unaffected by nicotine, (2) nicotine like activity formed in incubations cigarette smoking markedly inhibits human forearm. On the basis of thes vascular prostacyclin but not on p action by nicotine may facilitate pl reactive hyperemia. The data demo hyperemia in human skeletal mus reactive hyperemia, probably acts v inhibition of PG formation.

Wennmalm, Å. and Alster, P.

General Pharmacology 14(1):189-19

Other support: Swedish Tobacco C

From the Department of Clinical P Huddinge University Hospital, Huc

NICOTINE INHIBITS PROSTAG MICROSOMES

For this study of the influenc microsomal fractions of human ki patients, three men and three wom samples taken from the renal cortex and cortical and medullary microsc the absence and presence of nicotin methacin and with heat-inactivate diochromatograms of the renal co always displayed three major, and $PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. In additio some chromatograms of microsoma prostacyclin is the principal PG pro nicotine to the incubates resulted

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

NICOTINE INHIBITS VASCULAR PROSTACYCLIN BUT NOT PLATELET THROMBOXANE FORMATION

The metabolism of arachidonic acid (AA), a poly-unsaturated C:20 fatty acid, involves formation of a number of potentially active agents such as the primary prostaglandins (PG), thromboxane (Tx), prostacyclin (PGI_2), and the leukotrienes. In this paper, the metabolism of AA in two different tissues – platelets and vascular endothelium – is discussed with special reference to the effect elicited by nicotine on such metabolism. The effect of nicotine on the formation of Tx was studied in microsomes from human platelets, while the effect of nicotine on the formation of PGI_2-like activity was studied in rings of rabbit aorta incubated at room temperature in the absence or presence of nicotine or indomethacin. Results showed that (1) the conversion of [^{14}C]arachidonic acid to [^{14}C]thromboxane B_2 in rabbit or human platelet microsomes is unaffected by nicotine, (2) nicotine dose-relatedly inhibits the amount of prostacyclin-like activity formed in incubations of rabbit aortic rings or human veins, and (3) cigarette smoking markedly inhibits prostaglandin dependent reactive hyperemia in the human forearm. On the basis of these observations, it is concluded that nicotine acts on vascular prostacyclin but not on platelet thromboxane formation. Such a selective action by nicotine may facilitate platelet aggregability and counteract PG-dependent reactive hyperemia. The data demonstrate that (a) cigarette smoking inhibits reactive hyperemia in human skeletal muscle, and (b) cigarette smoking, when inhibiting reactive hyperemia, probably acts via the same mechanism as indomethacin, *i.e.*, via inhibition of PG formation.

Wennmalm, Å. and Alster, P.

General Pharmacology 14(1):189-191, 1983.

Other support: Swedish Tobacco Company.

From the Department of Clinical Physiology, Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

NICOTINE INHIBITS PROSTAGLANDIN SYNTHESIS IN HUMAN KIDNEY MICROSOMES

For this study of the influence of nicotine on prostaglandin (PG) synthesis in microsomal fractions of human kidney, samples were obtained from six informed patients, three men and three women, aged 42-61, undergoing nephrectomy. Tissue samples taken from the renal cortex and renal medulla were minced and homogenized, and cortical and medullary microsomal fractions were prepared and incubated both in the absence and presence of nicotine. Control incubations were carried out with indomethacin and with heat-inactivated microsomal fractions. Results showed that radiochromatograms of the renal cortical and renal medullary microsomal incubates always displayed three major, and well-defined, radiopeaks in parallel with 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. In addition, a low peak corresponding to PGD_2 appeared in some chromatograms of microsomal incubates of both tissues. These results reveal that prostacyclin is the principal PG product formed in human renal tissue. The addition of nicotine to the incubates resulted in a dose-related decrease in the amount of the

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

labelled PGs formed, and this effect was similar in both the renal cortical and renal medullary microsomal incubates. Indomethacin also induced a dose-related inhibition of the synthesis of [^{14}C]-PGs. However, indomethacin was much more efficient as an inhibitor of the [^{14}C]-PG formation, its potency in this respect being about 1000 times higher than that of nicotine. The fact that nicotine did not affect the distribution of the [^{14}C]-PGs formed suggests that the drug acted at the cyclo-oxygenase level, which is a step in synthesis common to all PGs. The authors of this paper suggest that the observed inhibitory effect of nicotine, even though less pronounced than that of indomethacin, deserves attention since it may provide a linkage between cigarette smoking and its sequelae in the cardiovascular system.

Alster, P. *et al. (Wennmalm, Å.)*

Acta Physiologica Scandinavica 117(4):581-583, 1983.

Other support: Swedish Tobacco Company, Swedish Medical Research Council, Magnus Bergvall Foundation, and the Karolinska Institutet, Stockholm.

From the Karolinska Institutet, Departments of Clinical Physiology, Pathology and Surgery, Huddinge Hospital, Huddinge, Sweden, and Clinical Physiology, St. Erik's Hospital, Stockholm.

EFFECT OF NICOTINE ON PROSTACYCLIN FORMATION IN RAT AORTA

The aim of the current study was to characterize the effect of nicotine on the conversion of arachidonate to prostacyclin (PGI_2) in the rat aorta. To do this, the effect of nicotine on the formation of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a metabolite of PGI_2 in the rat aorta, was investigated. Slices of rat aorta were incubated with [^{14}C]arachidonic acid ([^{14}C]AA) or ^{14}C-labeled prostaglandin endoperoxide H_2([^{14}C]PGH_2) in the presence of nicotine or indomethacin. ^{14}C-labeled products formed during the incubations were separated and quantified using thin layer chromatography and liquid scintillation spectrometry. A time- and substrate concentration-dependent formation of [^{14}C]6-keto-$PGF_{1\alpha}$ from [^{14}C]AA was found in the incubations. The K_M of AA for the formation of 6-keto-$PGF_{1\alpha}$ was 1.3×10^{-5} M. Nicotine competitively inhibited the formation of [^{14}C]6-keto-$PGF_{1\alpha}$ from [^{14}C]AA, the I_{50} being about 2×10^{-4} M. Indomethacin also decreased the formation of [^{14}C]keto-$PGF_{1\alpha}$ from [^{14}C]AA, with an I_{50} of about 10^{-6} M. Incubation of rat aorta slices with [^{14}C]PGH_2 also elicited the formation of [^{14}C]keto-$PGF_{1\alpha}$. This conversion was not affected by nicotine. It is concluded that nicotine inhibits the formation of PGI_2 in the rat aorta by competitive inhibition of the cyclo-oxygenase that converts arachidonate to prostaglandin endoperoxide. Furthermore, the data demonstrate that nicotine does not affect PGI_2 synthetase in this tissue.

Alster, P. and *Wennmalm, Å.*

European Journal of Pharmacology 86:441-446, 1983.

Other support: Swedish Tobacco Company and the Swedish Medical Council.

From the Department of Clinical Physiology at Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

VI. *Immunolo*

INHIBITION OF INTERFER(SUPPRESSOR (SIRS) MEDI/

Two reasonably well defi reported here; immunosuppres (IFNβ) or soluble immune resp activated murine T cells. SIRS SIRS, release a second factor, responsible for suppression. N peroxide can convert SIRS to M which produce a factor(s) with immune responses *in vivo* and i in clinically depressed hosts. T suppression were examined to g of this agent. SIRS or IFNβ su control responses: under optima of control levels in the presenc SIRS or Mø-SF directly, but inl conversion of SIRS to Mø-SF b sole inhibits SIRS- or IFNβ-me and suggest that some immunoc for by interference with nonspe

Aune, T. M. and Pierce, C. W.

International Journal of Immun

Other support: U. S. Public He

From the Department of Patholo St. Louis, and the Department Washington University School

MY-1, THE HUMAN MYELOI MONOCLONAL ANTIBODIE LACTO-*N*-FUCOPENTAOSE I

The anti-My-1 monoclonal mouse immunized with HL60 hu locytes and granulocytic precurs cytes, monocytes, platelets, or detected by this monoclonal antil cytic precursor cells. The sugar

Gal

which occurs in the glycolipid,

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

VI. *Immunology and Adaptive Mechanisms*

INHIBITION OF INTERFERON OR SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) MEDIATED SUPPRESSION BY LEVAMISOLE

Two reasonably well defined suppressor systems were employed in the studies reported here; immunosuppression was achieved with murine fibroblast interferon (IFNβ) or soluble immune response suppressor (SIRS), a product of concanavalin A-activated murine T cells. SIRS acts via macrophages (Mø) which after exposure to SIRS, release a second factor, Mø-derived suppressor factor (Mø-SF), that is directly responsible for suppression. Mø-SF appears to be modified SIRS since hydrogen peroxide can convert SIRS to Mø-SF directly. IFNβ also activates suppressor T cells which produce a factor(s) with properties identical to SIRS. Levamisole enhances immune responses *in vivo* and *in vitro* and is of value in improving immune responses in clinically depressed hosts. The effects of levamisole on IFNβ- or SIRS-mediated suppression were examined to gain a better understanding of the mechanism of action of this agent. SIRS or IFNβ suppressed plaque forming cell responses to 10-20% of control responses: under optimal conditions, these responses were increased to ~ 80% of control levels in the presence of levamisole. Levamisole did not inactivate IFNβ, SIRS or Mø-SF directly, but inhibited production of Mø-SF by SIRS-treated Mø and conversion of SIRS to Mø-SF by peroxide. These and other data indicate that levamisole inhibits SIRS- or IFNβ-mediated suppression by preventing formation of Mø-SF and suggest that some immunoenhancing properties of levamisole may be accounted for by interference with nonspecific suppressor T cell pathways.

Aune, T. M. and Pierce, C. W.

International Journal of Immunopharmacology 5(1):91-98, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology and Immunology, Washington University School of Medicine, St. Louis.

MY-1, THE HUMAN MYELOID-SPECIFIC ANTIGEN DETECTED BY MOUSE MONOCLONAL ANTIBODIES, IS A SUGAR SEQUENCE FOUND IN LACTO-*N*-FUCOPENTAOSE III

The anti-My-1 monoclonal antibody, produced by a hybridoma obtained from a mouse immunized with HL60 human promyelocytic leukemia cells, reacts with granulocytes and granulocytic precursor cells but not with normal peripheral blood lymphocytes, monocytes, platelets, or red cells. The cell surface antigen (designated My-1) detected by this monoclonal antibody is expressed on human granulocytes and granulocytic precursor cells. The sugar sequence

$$\begin{array}{l} \text{Gal}\beta\text{1-4GlcNAc}\beta\text{1-3Gal}\ldots. \\ \quad\quad\quad\

glycolipids, and in glycoproteins, contains the epitope recognized by anti-My-1 and 17 other monoclonal antibodies with the same cellular specificity. Although My-1 is strongly expressed only on granulocytic cells in the human hematopoietic system, it is also strongly expressed on some nonhematopoietic human and murine cells as well.

Huang, L. C., *Civin, C. I.*, Magnani, J. L., Shaper, J. H., and Ginsburg, V.

Blood 61(5):1020-1023, 1983.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; the Department of Pharmacology, University of Virginia, Charlottesville; and the Division of Pediatric Oncology, the Oncology Center, and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine, Baltimore.

STUDIES ON THE PATHOGENESIS OF THE ADULT RESPIRATORY DISTRESS SYNDROME

These studies of bronchoalveolar lavage (BAL) fluid from patients with adult respiratory distress syndrome (ARDS) were undertaken in an attempt to identify and characterize substances capable of activating components of the complement, contact, and clotting systems of plasma. Proteolytic activity of the fluids was observed by measuring cleavage of radiolabeled proteins of the contact (Hageman factor) and complement systems. Proteolytic activity was observed in 17 of 24 patients with ARDS, and BAL fluid of the seven ARDS patients without demonstrable, active enzyme exhibited inhibitory activity for the proteolytic activity. The enzymes cleaved Hageman factor, prekallikrein, plasminogen, high molecular weight kinogen, C4, C3, C5, and Factor B of the complement system. Cleavage of the contact system proteins produced fragments similar or identical in size to the fragments observed during activation of these molecules. None of seven normal individuals, and 29 of 99 patients with other forms of pulmonary disease contained measurable enzymes. Results showed that the proteolytic activity in BAL fluid of ARDS patients was blocked by diisopropylphosphofluoridate, Trasylol, soybean trypsin inhibitor, and normal plasma, or plasma deficient in inhibition of the first component of complement. Alpha$_1$-proteinase inhibitor (α1-PI)-deficient plasma failed to inhibit the proteolytic activity and addition of α1-PI to the deficient plasma reconstituted the inhibition. As seen here, much of the proteolytic activity of the BAL fluid from ARDS patients was identified as neutrophil elastase. In summary, the data presented here reveal that in BAL fluids from all 24 patients with ARDS, leukocytic elastase and/or α1-PI exist. A complex of elastase and α1-PI was observed in BAL fluids, and in some cases where active enzyme and α1-PI coexisted, free but inactive α1-PI was present.

McGuire, W. W., Spragg, R. G., Cohen, A. B., and *Cochrane, C. G.*

Journal of Clinical Investigation 69:543-553, 1982.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA; Pulmonary Division, University of California at San Diego, La Jolla, and the Pulmonary Division, Temple University Health Sciences Center, Philadelphia.

A PROTEASE-LIKE PERME
IMMUNOLOGIC IDENTITY

Extracts of guinea pig s
permeability factor, while act
creased vascular permeability
experiment presented here was
meability factor of skin extracts
showed that vascular permeabi
bility factor derived from guir
(αHFa) were both inhibited by
The permeability activity of b
F(ab')$_2$-Sepharose beads. The l
extracts produced a single im
gave a reaction of identity w
Hageman factor and anti-Hager
corrected the clotting activity of
activity was also blocked by ant
it was concluded that the skin p
ally indistinguishable from H
here, experiments using ^{125}I-H
Hageman factor in the skin ps
space in the harvested skin whi
space remains in question.

Yamamoto, T. and *Cochrane,*

American Journal of Patholog

Other support: National Institu

From the Department of Immu
Jolla, CA.

CELL-FREE TRANSLATION

In this attempt to cover f
elastin mRNAs, the methodolo
tion of RNA and preparation
reticulocyte lysate and optim
mRNAs; (c) identification of t
quantitation of tropoelastins in t
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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

A PROTEASE-LIKE PERMEABILITY FACTOR IN GUINEA PIG SKIN: IMMUNOLOGIC IDENTITY WITH PLASMA HAGEMAN FACTOR

Extracts of guinea pig skin have been shown previously to contain a potent permeability factor, while activated Hageman factor (HFa) is known to induce increased vascular permeability of considerable potency. With these things in mind, the experiment presented here was undertaken to determine specifically whether the permeability factor of skin extracts and Hageman factor are identical. Results of this study showed that vascular permeability enhancement activity of the protease-like permeability factor derived from guinea pig skin and of active guinea pig Hageman factor (αHFa) were both inhibited by anti-guinea pig Hageman factor rabbit $F(ab')_2$ antibody. The permeability activity of both factors was also absorbed on anti-Hageman factor $F(ab')_2$-Sepharose beads. The latent form of the permeability factor derived from skin extracts produced a single immunoprecipitation line with anti-Hageman factor and gave a reaction of identity with a precipitation band developing between purified Hageman factor and anti-Hageman factor. The latent permeability factor in the fraction corrected the clotting activity of Hageman-factor-deficient human plasma. The clotting activity was also blocked by anti-Hageman factor $F(ab')_2$ antibody. From these results, it was concluded that the skin permeability factor was immunologically and functionally indistinguishable from Hageman factor of plasma. In another study presented here, experiments using ^{125}I-Hageman factor showed that only 4 percent of the total Hageman factor in the skin pseudoglobulin fraction was derived from the vascular space in the harvested skin while the location of the Hageman factor in the interstitial space remains in question.

Yamamoto, T. and *Cochrane, C. G.*

American Journal of Pathology 107:127-134, 1982.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.

CELL-FREE TRANSLATION OF ELASTIN mRNAs

In this attempt to cover fully the system for effective cell-free translations of elastin mRNAs, the methodology is divided into four sections dealing with: (a) isolation of RNA and preparation of mRNA; (b) preparation of the mRNA-dependent reticulocyte lysate and optimal conditions for the cell-free translation of elastin mRNAs; (c) identification of tropoelastins in a cell-free translation assay; and (d) quantitation of tropoelastins in the cell-free translation system. Importantly, it is noted at this point that this discussion of the cell-free synthesis of elastin involves the synthesis of two proteins designated tropoelastins a and b. All the evidence to date suggests strongly that these two tropoelastins are separate gene products. Consequently, the methodologies described here are geared not only for the production of soluble elastin, but also for the separate identification of tropoelastins a and b. Sections of this paper are devoted to Isolation of Elastin mRNAs, Preparation of Lysate, Translation Conditions, Detection of Translated Tropoelastins, and Quantitation of Translated Tropoelastins, wherein rocket immunoelectrophoresis, a very sensitive technique for the direct quantitation of tropoelastins synthesized in the cell-free system, is described.

1002318995

Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

Foster, J. A. et al.

Methods in Enzymology 82:731-743, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

PREPARATION OF ANTISERUM TO TROPOELASTIN

The availability of antibody to soluble elastin is invaluable for detection, quantitation, and isolation of tropoelastin in a variety of *in vivo* and *in vitro* systems. The advantage to eliciting antiserum to tropoelastin is that the resulting antiserum contains precipitating antibody. The precipitin reaction between antibody and antigen is a prerequisite for performing various immunodiffusion and immunoelectrophoretic techniques available. In the preparation of antiserum, purified tropoelastin is injected intracutaneously into rabbits each week for a month. After a rest period of three weeks, another injection is given, and the rabbits are bled six-eight days later. The presence of precipitating antibody can be easily be tested by immunodiffusion. Specific antibody to tropoelastin can be prepared by affinity chromatography. The ideal ligand to attach onto a resin would be tropoelastin itself. However, because tropoelastin is very difficult to isolate in sufficient amounts, tryptic peptides released from lathyritic, insoluble elastin are an excellent alternative. In order to isolate tryptic peptides from the insoluble elastin, two different temperatures are used consecutively for trypsin digestion. In an attempt to bind specific tropoelastin antibody, 0.5 ml of chick tropoelastin antiserum is put onto a glass column and allowed to penetrate fully into a specifically prepared gel. The column is shut off for 15 minutes, then elution of serum proteins and nonelastin immunoglobulins are accomplished with 0.01 *M* Na_2HPO_4, 0.14 *M* NaCl(pH 7.4). Elution of the specific tropoelastin antibody is accomplished with 0.01 *M* sodium citrate, pH 2.8. It has been seen here that the antibody elutes immediately with this buffer and can easily be tested by immunodiffusion or immunoprecipitation.

Foster, J. A. et al.

Methods in Enzymology 82:762-765, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

ELASTIN GENE EXPRESSION

The scope of this article encompasses recent data relating to the molecular biology of elastin and the implications of these data to elastin biosynthesis. Since there are relatively few published reports in this area, a more general description of available techniques is presented, with a special emphasis on application to elastin. Included in this article are discussions of the isolation of elastin mRNAs, the optimal conditions for the cell-free translation of those mRNAs, procedures for the identification of elastin polypeptides directed by the synthesis of mRNA, the signal peptide found on the initially translated product, the differential expression of elastin mRNAs in various tissues during development, and the synthesis and cloning of elastin cDNA. Major sections of this paper deal with Isolation of Elastin mRNA; Cell-Free Translation of Elastin mRNAs; Identification of Tropoelastin; Tropoelastin Synthesis in Organ Cul-

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and B during Development; Eff
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Foster, J. A., Rich, C. B. and

International Review of Connec

Other support: National Institu

From the Department of Biolog

IgE-DEPENDENT AND IONO LEUKOTRIENES BY DOG M

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Phillips, M. J., *Gold, W. M.* an

The Journal of Immunology 131(

Other support: Medical Resea
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From the Howard Hughes Med
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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

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ture; The Signal Peptide of Tropoelastin B; Differential Expression of Tropoelastin A and B during Development; Effect of Glucocorticoids on Elastin Synthesis, and Synthesis and Cloning of Elastin cDNA. In conclusion, the data discussed regarding the translation of elastin mRNA, the regulation of this translation during development, and the current research involving the cloning of elastin cDNA fragments represent a good beginning to an understanding of elastin gene expression and regulation.

Foster, J. A., Rich, C. B. and Karr, S. R.

International Review of Connective Tissue Research 10:65-95, 1983.

Other support: National Institutes of Health.

From the Department of Biology, Syracuse University, Syracuse, NY.

IgE-DEPENDENT AND IONOPHORE-INDUCED GENERATION OF LEUKOTRIENES BY DOG MASTOCYTOMA CELLS

Dog mastocytoma cells sensitized with dog anti-ragweed IgE and challenged with ragweed antigen or stimulated with calcium ionophore A23187 generate LTC_4, lesser quantities of LTD_4 and LTB_4, and several mono-HETEs of which 12-HETE and 15-HETE are the principal products. In this study, the maximum production of leukotrienes evoked by antigen challenge was attained within 5 min, and was maintained for up to 45 min. In contrast, the maximum production of leukotrienes by mastocytoma cells activated with optimum concentrations of 0.2 μM to 1.0 μM calcium ionophore was realized only after 30 min at 37°C. The maximum levels of LTC_4 achieved by stimulation with calcium inophore were approximately 50% higher than those attained by antigen challenge, whereas the difference for LTB_4 was approximately 35%. Irrespective of the stimulus, the quantity of LTC_4 was 3 to 4 times greater than that of LTD_4 and 2 to 2½ times greater than that of LTB_4. The characteristics of generation of mono-HETEs by mastocytoma cells activated with calcium ionophore and antigen challenge were similar. The level of 12-HETE attained a maximum after 10 min and the quantities of 15-HETE and 5-HETE reached the highest levels after 30 min with both stimuli. The leukotriene products of mastocytoma cells were identified by high-performance liquid chromatography, spectral properties, and immunoreactivity with mono-specific antisera, and exhibited the same concentration-dependence of biologic activity as authentic synthetic standards. The rapid maximum response to IgE-dependent stimulation and the unique spectrum of products distinguishes the secretion of leukotrienes by dog mastocytoma cells from that by basophils and some other types of mast cells, and suggests diverse contributions of mast cell leukotrienes to immediate and late hypersensitivity reactions.

Phillips, M. J., *Gold, W. M.* and Goetzl, E. J.

The Journal of Immunology 131(2):906-910, 1983.

Other support: Medical Research Council of Great Britain, U. S. Public Health Service and the National Institutes of Health.

From the Howard Hughes Medical Institute Laboratories, Cardiovascular Research Institute, and Department of Medicine, University of California Medical Center, San Francisco.

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

STIMULUS SPECIFICITY OF THE GENERATION OF LEUKOTRIENES BY DOG MASTOCYTOMA CELLS

For the work presented here, the availability of dog mastocytoma cells of high purity that release histamine and leukotrienes (LT) B_4, C_4, and D_4 in response to antigen and ionophore A23187 has permitted an initial comparative analysis of the specificity of activation of the two compartments of mediators by distinct stimuli. Results showed that isolated dog mastocytoma cells sensitized with dog anti-ragweed IgE and challenged with ragweed antigen or incubated with ionophore A23187 or the carboxy-terminal dodecapeptide of platelet factor 4, PF4(59-70), release histamine and concurrently generate leukotrienes B_4, C_4, and D_4. In contrast, the exposure of mastocytoma cells to 0.1-3μg/ml of 15-hydroxyeicosatetraenoic acid (15-HETE) stimulates selectively the generation of leukotrienes, in the absence of histamine release, while 0.1-1 μg/ml of compound 48/80 releases histamine without enhancing the generation of leukotrienes. The observation that natural stimuli are capable of selectively activating one synthetic or secretory compartment of mast cells suggests that separate subsets of receptors as well as different biochemical events may serve to mobilize each class of mediators.

Goetzl, E. J., Phillips, M. J. and *Gold, W. M.*

Journal of Experimental Medicine 158:731-732, 1983.

Other support: National Institutes of Health, Medical Research Council of Great Britain and the U. S. Public Health Service.

From the Howard Hughes Medical Institute Laboratories, Cardiovascular Research Institute, and Department of Medicine, University of California Medical Center, San Francisco.

SERIAL CHANGES IN MARKERS OF DISEASE ACTIVITY WITH CORTICOSTEROID TREATMENT IN SARCOIDOSIS

Sarcoidosis is a systemic granulomatous disease of unknown cause that may lead either to pulmonary fibrosis and respiratory impairment or to spontaneous resolution. The great variability of the clinical course has made it difficult to assess clinically the effectiveness of corticosteroid treatment, the mainstay of therapy, on the disease process. Consequently, a variety of independent parameters of disease activity such as serum angiotensin-converting enzyme levels, ^{67}gallium lung scanning, and bronchoalveolar lavage have been recommended as useful adjuncts in the clinical management of patients with sarcoidosis. The study presented here was designed to prospectively assess changes in clinical status, pulmonary function studies, and routine simple clinical tests, such as measurements of serum gamma globulin levels and erythrocyte sedimentation rate, as well as more complex or less widely available studies, including ^{67}gallium lung scanning, measurement of serum angiotensin-converting enzyme levels, and bronchoalveolar lavage fluid analysis in a group of 12 patients with active disease treated with corticosteroids. While all individual parameters of disease activity improved statistically with therapy, there were individual differences among the patients as to which test(s) was the more reliable indicator. In general, changes in ^{67}gallium lung scanning scores most closely reflected changes in clinical status, although measurements of serum angiotensin-converting enzyme levels were also very reliable. However, the number of bronchoalveolar lavage fluid IgG-secreting cells

remained elevated in the absence o
bronchoalveolar lavage fluid lymph
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fluid analysis, in sarcoidosis.

Lawrence, E. C. et al.

The American Journal of Medicin

Other support: The American Lun
Kelsey-Seybold Foundation, and
Service.

From the Rockwell-Keough Pulm
Clinical Center of The Methodist H
College of Medicine, Houston.

REGULATORY PEPTIDES AND
RESPIRATORY TRACT OF MA

The early suggestion that a re
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Further verification is provided b
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Polak, J. M. and Bloom, S. R.

Experimental Lung Research 3(3&

From the Department of Histopath

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EURON SPECIFIC ENOLASE (
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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

remained elevated in the absence of clinically apparent disease, and the percentage of bronchoalveolar lavage fluid lymphocytes was often normal in patients who responded to corticosteriod therapy. Larger prospective studies are warranted to more extensively evaluate various measurements of disease activity, especially bronchoalveolar lavage fluid analysis, in sarcoidosis.

Lawrence, E. C. et al.

The American Journal of Medicine 74:747-756, 1983.

Other support: The American Lung Association, the National Institutes of Health, the Kelsey-Seybold Foundation, and the Veterans Administration Medical Research Service.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Center of The Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

REGULATORY PEPTIDES AND NEURON-SPECIFIC ENOLASE IN THE RESPIRATORY TRACT OF MAN AND OTHER MAMMALS

The early suggestion that a regulatory neuroendocrine system is present in the respiratory tract has now been shown to be a reality, confirmed by the finding of potently active peptides in both the autonomic nerves and mucosal APUD cells. Further verification is provided by the presence of a glycolytic enzyme, neuron-specific enolase (NSE), which can readily be immunostained to mark all components of this system in a single tissue section. The short review presented here deals with the distribution and cellular localization in the respiratory tract of five regulatory peptides (substance P, bombesin, vasoactive intestinal polypeptide (VIP), cholecystokinin, and somatostatin) and of the newly discovered neuroendocrine enzyme marker, NSE. Bombesin is found in typical mucosal endocrine cells, whereas the other regulatory peptides – principally substance P and VIP – are found, in significant concentrations, in autonomic nerves of the walls of the airways. Substance P, a putative sensory neurotransmitter, is found in autonomic nerves closely associated with the mucosal epithelium and the bronchial smooth muscle. VIP nerves, on the other hand, appear predominantly to innervate blood vessels, seromucous glands of the upper respiratory tract and bronchial smooth muscle. The presence of NSE in both mucosal APUD cells and autonomic nerves has established this neuronal enzyme as a useful marker for the entire neuroendocrine system of the lung and its derivative neoplasms.

Polak, J. M. and Bloom, S. R.

Experimental Lung Research 3(3&4):313-328, 1982.

From the Department of Histopathology, Hammersmith Hospital, London, England.

NEURON SPECIFIC ENOLASE (NSE) IMMUNOSTAINING A USEFUL TOOL FOR THE LIGHT MICROSCOPICAL DETECTION OF ENDOCRINE CELL HYPERPLASIA IN ADULT RATS EXPOSED TO ASBESTOS

As reported in this methodological paper, neuron specific enolase (NSE), an isoenzyme of the glycolytic enzyme enolase, has been immunostained in the endocrine

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

cells of the lung. Application of this method at light microscopical level clearly shows endocrine cell hyperplasia in the lungs of adult rats exposed to asbestos. Specifically, a marked endocrine cell hyperplasia at light microscopical level was revealed in the lungs of adult rats exposed to asbestos using antibodies to NSE. Very large groups of NSE-immunoreactive cells (20-80) were only observed in the lungs of rats exposed to asbestos for 12 months. In addition, smaller groups of cells (2-10) known to be present normally and to decrease with age, were rarely noted in the controls but were detected frequently in the treated rats. Therefore, immunoreactive NSE is a very good marker for endocrine cell hyperplasia and of early neoplastic changes.

Sheppard, M. N., Johnson, N. F., Cole, G. A., Bloom, S. R., Marangos, P. J., and *Polak, J. M.*

Histochemistry 74:505-513, 1982.

Other support: Wellcome Trust and the M.R.C. (UK).

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London; Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, Wales; and the Clinical Psychobiology Branch, National Institute of Mental Health, Bethesda, MD.

DISTRIBUTION OF REGULATORY PEPTIDES IN THE RESPIRATORY TRACT OF MAN AND MAMMALS

For the review presented here, the distribution of five regulatory peptides, vasoactive intestinal polypeptide (VIP), substance P, bombesin, cholecystokinin (CCK), and somatostatin, was investigated in man (adult and neonate) and in mammals (guinea pig, rat and cat) using immunocytochemistry and radioimmunoassay. Results showed that VIP and substance P, localized by immunocytochemistry to the lung innervation, were the most abundantly distributed. Bombesin, mainly localized in mucosal endocrine cells, was present in somewhat lower concentrations. The quantities of somatostatin and CCK were below the limits of immunocytochemical detection. The characteristic pattern of distribution of VIP and substance P in the lung innervation, and their proposed origin, is in keeping with their sets of actions. VIP is known to be a vasodilator, muscle relaxant and secretomotor neurotransmitter, whereas substance P, contained in nerve fibers that are shown in this study to originate from primary sensory neurons of the nodose ganglion, is regarded as a sensory neurotransmitter. In addition, some lung endocrine tumors were shown to contain significantly large concentrations of regulatory peptides, in particular bombesin, which was found to be present in numerous oat cell carcinomas. In conclusion, the use of antibodies to neuron-specific enolase is proposed, in this study, as the best tool for the visualization of the diffuse neuroendocrine system in its entirety, both in normal tissues and in disease states, particularly neuroendocrine neoplasias.

Polak, J. M. and Bloom, S. R.

In: Bloom, S. R., Polak, J. M. and Lindenlaub, E. (eds.): *Systemic Role of Regulatory Peptides*, New York: F. K. Schattauer Verlag, 1982, pp. 241-269.

Other support: The Wellcome Trust, UK.

From the Department of Histopathology, Hammersmith Hospital, London, England

IMMUNOCYTOCHEMICAL
ISLETS OF LANGERHANS

Previous radioimmunob
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Alejandro, R., Shienvold, F.

Diabetes 31(suppl. 4):17-22,

Other support: National Inst
tion, and Veterans Administ

From the Departments of M
Medicine, Miami, FL.

TISSUE CULTURE REDU
ISLETS AND PROLONGS

In this study it is shown
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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

IMMUNOCYTOCHEMICAL LOCALIZATION OF HLA-DR IN HUMAN ISLETS OF LANGERHANS

Previous radioimmunobinding assay studies have shown that human islet-enriched fractions possess HLS-A, -B, -C and DR antigens and that they are capable of eliciting allogeneic lymphoproliferative responses. The present study was undertaken to identify directly the cell type(s) that carries DR antigens in human islets, using ultrastructural immunocytochemistry with a nonpolymorphic monoclonal anti-DR antibody. In this study the presence of DR in the islet-enriched fractions (IEFs) was first demonstrated by radioimmunobinding assay. Light microscopic immunocytochemistry, in frozen sections of intact (unfixed) human pancreas, revealed a staining pattern suggestive of a vascular distribution of DR in islets. Ultrastructural localization of DR was then carried out by indirect immunoperoxidase labeling in the presence of NaN_3 (to prevent internalization of bound αDR). The major site of DR expression in the islet was the endothelial cell surface. Endocrine cells were devoid of αDR binding. Nonislet endothelium was also heavily labeled, but acinar and ductal cells were completely negative. Leukocytes bound αDR but were relatively rare in the IEFs. Human islets, therefore, clearly express HLA-DR, but predominantly on insular endothelial cells. Isolation of pure endocrine cell populations specifically free of endothelium would appear to be a rational approach to reducing immunogenicity in allogeneic transplantation.

Alejandro, R., Shienvold, F. L., Hajek, S. V., *Ryan, U.*, Miller, J., and Mintz, D. H.

Diabetes 31(suppl. 4):17-22, 1982.

Other support: National Institutes of Health, the Diabetes Research Institute Foundation, and Veterans Administration Research Support.

From the Departments of Medicine and Surgery, University of Miami School of Medicine, Miami, FL.

TISSUE CULTURE REDUCES Ia ANTIGEN-BEARING CELLS IN RAT ISLETS AND PROLONGS ISLET ALLOGRAFT SURVIVAL

In this study it is shown that lymphocytes, macrophages and capillary endothelial cells in rat islets of Langerhans bear Ia antigenic determinants, that these cells are reduced by culture, and that this is accompanied by a significant decrease of Ia antigen density as determined in a sensitive radioimmunobinding assay. To be precise, after it was shown here that rat pancreatic islet allograft survival was prolonged by a prior 7-day period of tissue culture, attention was drawn to identifying those cells in islets capable of stimulating allograft rejection (Ia antigen-bearing cells) and to determining whether such cells and/or their Ia antigens might be reduced by tissue culture. Freshly isolated and 7-day-cultured Wistar-Furth rat islets were incubated with a mouse anti-rat Ia nonpolymorphic monoclonal antibody, then with peroxidase-conjugated goat anti-mouse antibody, and processed for electron microscopy. Peroxidase (Ia)-positive lymphocytes, macrophages, and capillary endothelial cells were identified in fresh but not in cultured islets. A radioligand assay, using ^{125}I-protein A, revealed a 45% decrease in binding of Ia antibody to cultured compared with fresh islet cells. It was concluded, therefore, that Ia antigen-bearing lymphocytes, macrophages, and capil-

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000

lary endothelial cells in rat islets are reduced by tissue culture and that this may account, at least in part, for the decreased immunogenicity of cultured islet allografts.

Rabinovitch, A., Alejandro, R., Noel, J., Brunschwig, J-P., and *Ryan, U. S.*

Diabetes 31(suppl. 4):48-54, 1982.

Other support: National Institutes of Health, NIAMDD Institutional Training Grant, and the Diabetes Research Institute Foundation.

From the Division of Endocrinology and Metabolism, the Department of Medicine, and the Department of Pharmacology, University of Miami School of Medicine, Miami, FL.

MATHEMATICAL ANALYSIS OF ENDOTHELIAL SIBLING PAIR CELL-CELL INTERACTIONS USING TIME-LAPSE CINEMATOGRAPHY DATA

In the study presented here, the division and migration behavior of endothelial cells under two differing culture conditions were analyzed by time-lapse cinematography. It was seen that in response to mechanical wounding of an intact monolayer, a relatively small proportion of the remaining cells immediately divide and migrate to fill the denuded area. However, the rates of division and migration of these cells are higher than for cells in a regular culture during growth to confluency. In addition in the wounded culture, sibling pairs tend to migrate in parallel and divide in synchrony. As repair of the wound proceeds, the migration behavior of cells in the wounded culture takes on characteristics of the regular culture, *e.g.* siblings migrating in opposite directions. As confluency is reached in both cultures cell division and migration cease. It was significant that in the wounded culture the first generation of siblings were very close (less than 150 μm apart) at division. Overall, the behavior differences between the two cultures resulted in a higher rate of increase in cell numbers, and thus faster repair, of the wounded monolayer.

Brown, L. M., *Ryan, U. S.*, Absher, M., and Olazabal, B. M.

Tissue & Cell 14(4):651-655, 1982.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL; Newnham College, Cambridge, England, and the Department of Medicine, University of Vermont School of Medicine, Burlington.

A SINGLE MONOCLONAL ANTI-Ia ANTIBODY INHIBITS ANTIGEN-SPECIFIC T CELL PROLIFERATION CONTROLLED BY DISTINCT Ir GENES MAPPING IN DIFFERENT H-2 I SUBREGIONS

Monoclonal antibodies (MAb), which are powerful reagents for both preparative and analytic purposes, have proven extremely useful in experiments aimed at elucidating the molecular basis of immune response (Ir) gene function and the mechanism of Ia-restricted T cell activation. In the work reported here, a xenogeneic rat anti-mouse Ia monoclonal antibody, M5/114 ($\gamma 2b,\kappa$), was studied for its effects *in vitro* on T cell

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Germain, R. N., Bhattachar

The Journal of Immunology

Other support: National Ins

From the Department of P
chemistry, Sidney Farber Ca

MAC-1 ANTIGEN: QUANT POPULATIONS AND TISS LOCALIZATION IN SPLEI

As reported in this pap
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that Mac-1 is synthesized by m
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Ho, M-K. and *Springer, T. A*

The Journal of Immunology

Other support: U. S. Public

From the Laboratory of Memb
Harvard Medical School, Bos

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Source: https://www.industrydocuments.ucsf.edu/docs/gqhk0000